IN-VITRO INHALATION PERFORMANCE FOR FORMOTEROL DRY POWDER AND METRED DOSE INHALERS

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In-vitro inhalation performance for formoterol dry powder and metred dose inhalers

In-vitro characteristics of the emitted dose from the formoterol dry powder and metred dose inhalers to identify the influence of inhalation flow, inhalation volume and the number of inhalation per dose

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Key words: Formoterol fumarate, Atimos Modulite, Turbuhaler, Easyhaler, Foradil Aeroliser, dose emission, lung deposition.

Abstract

The present work aimed at assessing the dose emission and aerodynamic particle size characteristics of formoterol fumarate from Atimos Modullite, a metered dose inhaler (MDI) and Foradil Aeroliser, Easyhaler, and Oxis Turbuhaler dry powder inhalers (DPI) at different inhalation flow rates and volumes using in vitro methodology. Recognised methods have been adopted and validated to generate the results.

The in vitro characteristics of formoterol were measured according to standard pharmacopeial methodology with adaptation to simulate routine patient use. The dose emission from the Atimos Modulite was determined using inhalation volumes of 4 and 2 L and inhalation flows of 10, 28.3, 60, and 90 L/min. The %nominal dose emitted was consistent between the various flow rates and inhalation volumes of 4 and 2L. The particle size distribution was measured using an Anderson Cascade Impactor (ACI) combined with a mixing inlet valve to measure particle size distribution at inhalation flow rates below 30 L/min. The particle size distribution of formoterol from Atimos Modulite was measured using inhalation flows of 15, 28.3, 50, and 60 L/min with and without different spacers, Aerochamber and Volumatic. The mean fine particle dose (%nominal dose) through an Atimos without spacer were 53.52% (2.51), 54.1% (0.79), 53.37% (0.81), 50.43% (1.92) compared to Aerochamber 63.62% (0.44), 63.86% (0.72), 64.72% (0.47), 59.96% (1.97) and Volumatic 62.40% (0.28),63.41% (0.52), 64.71% (0.61), 58.43% (0.73), respectively. A small decrease in the fine particle dose was observed as the inhalation flow increased, but this was not significant. The respective mean mass aerodynamic diameter (MMAD) increased as the flow rate was increased from 15 of 60 L/min. Results also suggests that the use of spacers provides better lung deposition for patients with problems using MDI.

The dose emission from the Foradil Aeroliser was determined using inhalation volumes of 4 and 2 L, at inhalation flows of 10, 15, 20, 28.3, 60, and 90 L/min plus two inhalations per single dose. The %nominal dose emitted using 2 L inhalation volume was approximately half when compared to results obtained using inhalation volume of 4 L. A significantly ($p<0.001$) higher amount of drug was also emitted from Easyhaler® at inhalation volume of 4 L through flow rates of 10, 20, 28.3, 40, and 60 L/min compared 2 L. Similar results were observed through Oxis Turbuhaler at inhalation flow rates of 10, 20, 28.3, 40, and 60 L/min.

Comparative studies were also carried out to evaluate the particle size distribution of formoterol through the DPIs. The nominal fine particle dose through Aeroliser using inhalation flows of 10, 20, 28.3, 60 and 90 L/min were 9.23%, 14.70 %, 21.37%, 28.93%, and 39.70% for the 4 L and 4.17%, 5.55%, 7.28%, 8.41%, and 11.08% for the 2 L, respectively. The respective MMAD significantly ($p<0.001$) decreased with increasing flow rates. Aeroliser performance showed significant ($p<0.001$) increase in the % nominal fine particle dose for two inhalations compared to one inhalation at both 4 and 2 L.

The Easyhaler was measured using inhalation flows of 10, 20, 28.3, 40, 60 L/min. The nominal fine particle dose were 19.03%, 27.09%, 36.89%, 49.71% and 49.25% for the 4 L and 9.14%, 15.44%, 21.02%, 29.41%, 29.14% for the 2 L, respectively. The respective MMAD significantly ($p<0.001$) decreased with increasing flow rates. Easyhaler performance at both 4 and 2 L showed no significant differences between one and two inhalations at low flow rates (10, 20, 28.3), but this was significant ($p<0.05$) at higher flow rates (40 and 60 L/min).

The Oxis Turbuhaler was also measured using inhalation flows of 10, 20, 28.3, 40, 60 L/min. The nominal fine particle dose were 12.87%, 24.51%, 28.25%, 34.61%, 40.53% for the 4 L and 8.55%, 15.31%, 21.36%, 19.53%, 22.31% for the 2 L, respectively. Turbuhaler performance showed significant ($p<0.05$) differences between one and two inhalations at varying flow rates 2 L inhalation volumes, but not at 4 L.

The use of Foradil Aeroliser delivers small particles as the Oxis Turbuhaler using two inhalations hence delivering formoterol deep into the lungs. Also, this thesis shows that high flow resistance of Turbuhaler will indeed influence the ability of patients with severe asthma or children to use the system. Beside, Easyhaler produced the highest drug delivery to the lungs, thus, making it a more desirable system to use, especially for children and asthma sufferers.

Section of thesis have already been published in the following form

1- Alaboud S, Assi KH, Chrystyn H. In-vitro characterisation of the emitted dose from the Foradil Aerolizer (DPI) to identify the influence of inhalation flow, inhalation volume and the number of inhalations per dose. Respiratory Drug Delivery (RDD®) Sience Conference, Orlando, Florida, April 25-29, 2010.

2- Alaboud S, Assi KH, Chrystyn H. IN-VITRO CHRACTERAISATION OF THE EMITTED DOSE FROM FORADIL AEROLIZER (DPI) TO IDENTIFY THE INFLUENCE OF INHALATION FLOW AND INHALATION VOLUME. School Life Sciences (SLS). University of Bradford, Poster day, Wednesday 23 March 2010

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DEDICATION

This dissertation is dedicated with affection to the lights of my life to the soul of my father and mother, for their endless love.

Abbreviations

ACI	Andersen cascade impactor
AMP	Adenosine monophosphate
ATP	Adenosine triphosphate
BP	British Pharmacopeia
BTS	British Thoracic Society
CFC	Chorofluocarbon
COPD	Chronic obstructive pulmonary disease
DPI	Dry powder inhalers
EP	European Pharmacopeia
ECG	Electrocardiogram
FEV	Forced expiratory volume in one second
FPD	Fine particle dose
FPF	Fine particle fraction
GSD	Geometric standard deviation
HFA	Hydrofluoroalkane
HPLC	High Performance Liquid Chromatography
ICS	Inhaled corticosteroid
MMAD	Mass median aerodynamic diameter
NICE	National Institute for Health and Clinical Excellence
NGI	Next generation impactor
pMDI	Pressurised metered dose inhaler
PEFR	Peak expiratory flow rate
PET	Positron emission tomography
TED	Total emitted dose
USP	United States Pharmacopeia

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CHAPTER ONE

INTRODUCTION

1.0 INTRODUCTION

1.1 Background

The human respiratory system pathway starts at the nostrils and mouth through which air enters passing through the nasopharynx (nostrils only), the oral pharynx, and the glottis. The air subsequently travels through the trachea into the right and left bronchi, which branches and re-branches into the bronchioles. Each one of these branches terminates in a cluster of alveoli where the actual gas exchange takes place (Hickney, 1999).

Asthma and chronic obstructive pulmonary disease (COPD) are respiratory disorders that emerge as a result of irritants reaching the bronchi and bronchioles and stimulating an increased secretion of mucous, inflammation, and airway damage. Asthma is a result of the airways response to an allergen. Emphysema and chronic bronchitis commonly occur together and are known as COPD but can also occur separately.

Asthma and COPD can be treated systemically or by local administration of drugs (bronchodilator and corticosteroids) to the lungs via the inhaled route. The inhaled route of administration is the preferred route for delivering bronchodilators and corticosteroids to patients with asthma and COPD. The inhaled route allows delivery of a small but therapeutic dose of drug directly to the airways thereby achieving a high local concentration within the lung, while at the same time minimising side effects of the drug due to lower systemic delivery compared to an equivalent oral dose.

Central to the success of inhaler treatment has been the availability of aerosol delivery systems or inhalers (Chrystyn, 2006). There are three most popularly used inhalation systems. nebulisers, pressurised metered dose inhalers (pMDIs), and dry powder inhalers (DPIs) that have provided a major advancement in the treatment of respiratory disorders including asthma and COPD Neubulisers form a fine mist of drug particles

from a solution, which are inhaled by patients who have difficulty using inhalers such as in cases of serious respiratory diseases or acute exacerbations (Decker, 2004). Neubulisers tend to be used in hospital settings for patients.

pMDIs are the most common inhalation device used worldwide. The inhaled bronchodilators and inhaled corticosteroids (ICS) have all been formulated in pMDIs because they are small, cheap, portable, and deliver consistent doses up to labelled claim (BTS, 1997). On the other hand, pMDIs have many drawbacks. These include the difficulty for patients to inhale simultaneously as the dose is released from the device causing the amount of the medication depositing in the back of the mouth or on the tongue, thus reducing effectiveness and poor compliance (Crompton, 1982). This drawback to the device was addressed with the introduction of pMDIs add-on devices referred as spacers and breath-operated pMDIs (O'Callaghan et al, 1997). All pMDIs consist of four basic functional elements which are the container, the metering valve, the actuator, and the mouthpiece. Their formulations contain a liquified propellant, which provides an energy source to expel the formulation from the valve in form of rapid evaporating droplets and as a dispersion medium for the drug and other excipients (Hickey, 1996). For pMDIs the inhalation maneuver is the most relevant element for deposition efficacy. Common problems as well as poor synchronisation between actuation and inhalation include stopping inhalation when the aerosol hits the back of the throat (cold freeze effect) and inhaling too fast. This leads to decreased lung deposition and increased oropharyngeal deposition of the drug, resulting in a decreased therapeutic benefit and increased local and systemic side effects (Virchow et al, 2007). Thus, hand-lung coordination and slow inhalation are of major importance during the routine use of pMDIs.

DPIs were first introduced in the mid 1980's and became increasingly popular following the Montreal Protocol in 1987 on the phasing out of chorofluocarbon (CFC) propellant in pMDIs inhaler together with the realisation of the potential for high lung deposition from DPIs. However, phasing out of the CFC propellants has created formulation difficulties as there are solubility problems with new propellants like HFA (Hydrofluoroalkanes). DPIs can be divided into two major groups, the single dose DPIs including Aeroliser®, Handyhaler®, and Rotahaler®, and the multi-dose inhalers DPIs including Turbuhaler®, Clickhaler®, and Diskhaler®. Most DPIs contain micronised drug blended with larger carrier particles, which prevent aggregation of small particles and helps flow.

Due to the inhaler design and DPI formulation and its properties, the fine particle output depends more strongly on patient's inhalation flow for drug delivery to the lungs. The patient's inhalation flow interacts with the resistance inside the DPI to generate a turbulent energy which de-aggregates the formulation into an emitted dose containing particles that have the potential for lung deposition (Chrystyn, 2003). The dose emitted from an inhaler device with particles with a size <5μm that have the potential to deposit in the lungs are known as fine particle dose. All DPIs have different internal resistance (Chrystyn, 2003), which differs in the inhalation flow used by a patient. As a result, the fine particle output is very much flow dependent with some DPIs more prone to this than others (Chrystyn, 2003). The faster the inhalation flow through a DPI then the greater will be the turbulent energy and therefore the better is the quality of the emitted dose. However, studies have highlighted that some patients with very severe lung dysfunction cannot inhale at the desired flow in order to achieve the optimum dose during routine use with a DPI (Chrystyn, 2003; Pedersen et al, 1986; Pedersen and Steffensen, 1986).These studies have also revealed that young children are also very likely to have problems using the fast inhalation flow.

Different inhalers due to formulations and device designs provide varying degrees of resistance to inhalation flow and require different inhalation techniques as in the way which the patient uses the inhaler. All current commercially available DPI devices are flow dependent. pMDIs are used with a flow as low as possible (Newman 1982) while DPIs deliver a larger pulmonary dose at a high inhalation flow (Newman et al, 1991; Borsgstrom 1994; Pitcairn et al, 1994).

Bisgaard and co-workers (1998) have studied the range of particle sizes recovered from some DPI devices and they have categorised the particle sizes as 'large particles' (>4.7μm), 'fine particles' (<4.7μm), and 'very fine particles' (<2.1μm). The study also revealed that devices with the highest proportion of particles in the middle range had the greatest therapeutic effect (Bisggard et al, 1998). In a similar study, it was demonstrated that a particle size of 3μm has an optimum clinical effect than particles of size particle 1.5μm and 5μm, which induced significantly less bronchodilation (Zanen et al, 1995).

Many patients experience problems using their devices correctly. Poor inhalation technique can markedly reduce the proportion of the drug that reaches the lung. Studies suggest that 28-68% of the patients with asthma have problems using their pMDIs and DPIs sufficiently well to benefit from the dose (Raul, 2006). It has been reported that 92% of patient that uses a MDI either demonstrated poor coordination on first inhalation flow or both first and second inhalation (Raid et al, 2007). Overall, the issue of correct use of inhalers is of critical importance in maintaining optimal asthma control as patients who use their inhalers incorrectly tend to have less stable asthma than those who use their device correctly (Giraud and Roache, 2002). For DPIs the Pharmacopeias (USP, 2009; EP, 2007, BP, 2008) recommended the use of an inhalation volume of 4 L at a constant flow corresponding to a pressure drop of 4 KPa across the inhaler to measure both the total emitted dose and the aerodynamic characteristics of the emitted

dose. However, in routine practice patients use varying flow and volumes due to variety of factors such as lung size (age and gender), degree of airway obstruction that is present and inspiratory musculature (Chrystyn 2004, Stocks, 1995). Furthermore, when patients inhale through a DPI, the inhalation volume is less than 4 L, for example, it has been reported that the inhaled volume for asthmatics and COPD patients is about 2 L (Hawskworth et al, 2000).

The aerodynamic particle characteristics of spray discharged from pMDI or from a powder cloud from a DPI is an important characteristic in judging inhaler performance and ensuring effective drug delivery to the lungs. This is characterised by three parameters: the mass median aerodynamic diameter (MMAD), the fine particle dose (FPD), and the geometric standard deviation (GSD). The MMAD measures the diameter of particles as a function of density and velocity. The FPD is the 'respirable' dose (particles with a MMAD <5 μm) that represent a theoretical amount that has the greatest potential for lung deposition. The GSD is a measure of the polydisperse nature of the aerosol particles. These parameters are utilised in industry as quality control measures to compare the equivalence of different inhaler devices and ensure that inhalation products are likely to be clinically effective (Taylor, 2002).

To measure the in-vitro characteristics of the emitted dose, the Anderson Cascade Impactor (ACI) is one of the method described in the United States Pharmacopeia (USP, 2007), European Pharmacopeia (EP, 2007) and British Pharmacopeia (BP, 2008). Traditionally, the ACI has been designed to operate at a flow of 28.3 L/min, but are now available to measure at 60 or 90 L/min. Since, patients inhale at different flows so the use of different flows will alter the cut-off diameter of each stage of the ACI. To overcome this problem, modifications to the ACI with the use of a mixing inlet valve has recently been introduced which enabled the measurement of the characteristics of

the emitted dose from DPIs at a variety of flows consistent with the patient's routine use (Nadarassan et al, 2010).

There is very little information available in the literature about the flow dependent properties of pMDIs. However, studies have been carried out in which inspiratory flow rate was controlled and drug deposition and clinical outcome were compared between PMDI and DPI. In such studies results demonstrated the importance of inspiratory flow rate on the performance characteristics of DPI in the clinical outcome (Pedersen et al, 1990, Engel, 1989, Engel et al, 1992, Dolovich et al, 1988). On the other hand, PMDI demonstrated more reproducible dosing characteristics independent of inhalation flow rate (Smith and Schultz, 1996). Moreover, studies by Newman et al (1995) have compared the deposition of terbutaline from pMDIs using gamma scintigraphic technique. The volunteers were trained to inhale slow (27 L/min) and fast (151 L/min) using the pMDI. Better deposition was reported by volunteers inhaling at slow rate, same time as they press down on the medication canister. An in vitro method using ACI and mixing inlet valve has also been designed to study the effect of deposition of particles from DPIs at low flows (Nadarassan et al 2009).

1.2 Critical Justification of Work

pMDIs have long been used for the treatment of asthma and COPD. The phasing out of CFCs propellant and using HFAs has introduced a new challenge for the pharmaceutical industries. Most drugs are insoluble in HFAs and drugs that solubilise are unstable. Until recently no formoterol pMDI was available due to formulation and stability issues. However, Atimos® Modulite® (Trinity-Chiesi) has recently been launched formulated in a pMDI containing HFA propellants and 12 μg dose of formoterol. The Modulite technology (Ganderton et al, 2002) used permutes the following variables: non-volatile

components of a solution formula, the actuator orifice geometry, the volume of the metered solution and the vapour pressure of the propellants. This permits the design of aerosols with chosen particle size and plume speed. This facilitates coordination of the dose generation with inspiration, reduces oropharyngeal. Very little is known about the delivery of particles from the Modulite formulation of formoterol in a pMDI at different inhalation flows, which has been extensively studied in this research programme.

DPI devices have also been studied. Chen et al (2001) reported a comparative study between a Foradil® Aeroliser® and Oxis® Turbuhaler®, a four-stage liquid impinge (Copley, Nottingham, UK) with a glass throat was used in this study. The results revealed that at higher air flows (90 and 120 L/min) greater amounts of the fine particles were generated (3.9 and 4.1 μg) using Foradil® Aeroliser® and Oxis® Tubuhaler®. Also Nora (2001) reported that the fine mass was approximately 30% of the label claims of 12 μg for both inhalers.

Another comparative study on Foradil® Aeroliser® and Oxis® Turbuhaler® using the eight-stage Anderson Cascade Impactor was performed to determine the fine particle dose and the MMAD at varying flows (28.3, 30, 60, 80 L/min) (Weuthen et al, 2002). The effect of low flows (<28.3 L/min) on fine particle deposition has not been reported. However, many patients with COPD cannot achieve an inhalation flow of 28.3 L/min or lower through the Turbuhaler® (Pedersen, 1994).

Nevertheless, recent studies by Nadarassan et al (2009) have demonstrated for the first time the dose emission characteristics of Turbuhaler® at different flows at inhalation volumes 4 L using mixing inlet valve. Such methodological approach revealed a steep increase in the percentage of fine particle dose as the flow increases to 60 L/min (Nadarassan et al, 2009). However, this study did not include the particle distribution profile whilst using different inhalation volumes of 2 L and 4 L.

The purpose of the work will be to carry out a detailed in-vitro analysis for four inhalation devices: Foradil® Aeroliser®, Oxis® Turbuhaler®, Formoterol Easyhaler® (DPIs) and Modulite® (pMDI) currently available for Formoterol. The BP and EP (2006) also recommend an inhalation volume of 4 L across the device but patients with severe lung disorders cannot achieve the 4 L (Hawksworth et al, 2000). Thus it is necessary to understand the effect on the aerodynamic particle profile when different inhalation volumes are used.

1.3 Aim

The aims of this study were to:

- Assess by in vitro methods the effects of different inhalation flows and inhalation volumes on the aerodynamic characteristics of the emitted dose of formoterol from the Atimos® Modulite® (pMDI).
- Evaluate and compare by in-vitro methods the effects of different inhalation flows and inhalation volumes on the aerodynamic characteristics of the emitted dose from the Foradil Aeroliser®, Oxis Turbuhaler®, Formoterol Easyhaler® (DPIs), when inhaling once or twice per dose.

1.4 Objectives

1. To measure the effect of flow on the dose emission and dose content uniformity of a formoterol Atimos® Modulite® at flows of 10. 28.3, 60, and 90 L/min using inhalation volumes of 2L and 4 L.

2. To evaluate the particle size distribution for dose emitted from the Atimos® Modulite® with and without a spacer at flows of 15, 28.3, 50 and 60 L/min using mixing inlet.
3. To measure the effect of flow on the dose emission of Foradil Aeroliser®, Easyhaler®, and Turbuhaler® at flows of 10, 15, 20, 28.3, 40, 60 and 90 L/min using inhalation volumes of 2 L and 4 L as well as the effect of using one and two inhalations per dose.
4. To evaluate the particle size distribution for the dose emitted from the Foradil® Aeroliser® using inhalation volumes of 2 L and 4 L at flows of 10, 20, 28.3, 60, and 90 L/min as well as the effect of using one and two inhalations per dose.
5. To determine the aerodynamic properties of the dose emitted from a formoterol Easyhaler® at flows of 10, 20, 28.3, 40, and 60 L/min using the mixing inlet at inhalation volumes of 2 L and 4 L as well as the effect of using one and two inhalations per dose.
6. To determine the aerodynamic properties of the dose emitted from Oxis Turbuhaler® at flows of 10, 20, 28.3, 40, and 60 L/min using the mixing inlet valve at inhalation volumes of 2 L and 4 L as well as the effect of using one and two inhalations per dose.

1.5 Thesis structure

This thesis consists of seven chapters:

Chapter one is a general introduction explaining the types of inhalers and the principles governing the operation of aerosol delivery devices, especially pMDIs and DPIs. The

factors that affect the delivery and deposition of the aerolised drug particles to the target site in the lungs. Previous studies on the effect of inhalation flows, inhalation volume and one or two inhalations on dose emission of a single dose capsule type inhalers have been highlighted. Also, the aims and objectives of the research have been included.

Chapter two is the literature review of the issues related to this research including:

- The respiratory system including structure of anatomy.
- Asthma and COPD and their therapeutic management.
- Inhalation therapies: introduction of drug delivery system and mechanisms of particle deposition, methods to determine the bioequivalence of inhaled products.

Chapter three describes the materials, methods, instrumentation, and methodology used for High Performance Liquid Chromatography (HPLC).

Chapter four is the in vitro determination of the dose emission of formoterol from Foradil Aeroliser®, Oxis Turbuhaler®, and Easyhaler® at different flow rates using inhalation volumes of 2 L and 4 L.

Chapter five describes the in-vitro determination of the particle size characteristics of the emitted dose of formoterol from Foradil Aeroliser®, Oxis Turbuhaler®, Formoterol Easyhaler® inhalers at varying inhalation flows at inhalation volumes of 2 L and 4 L using one and two inhalations per dose.

Chapter six is the determination of particle size characteristics of the emitted dose from Atimos Modulite inhalers at varying inhalation flows and the effect of using a spacer.

Chapter seven includes a general discussion and conclusion.

Chapter eight references.

CHAPTER TWO

LITERATURE REVIEW

2. LITERATURE REVIEW

2.1 Respiratory System

The human respiratory tract is a branching system of air channels with more than 23 bifurcations, which initiates from the mouth and terminates in a cluster of alveoli (Figure 2.1).

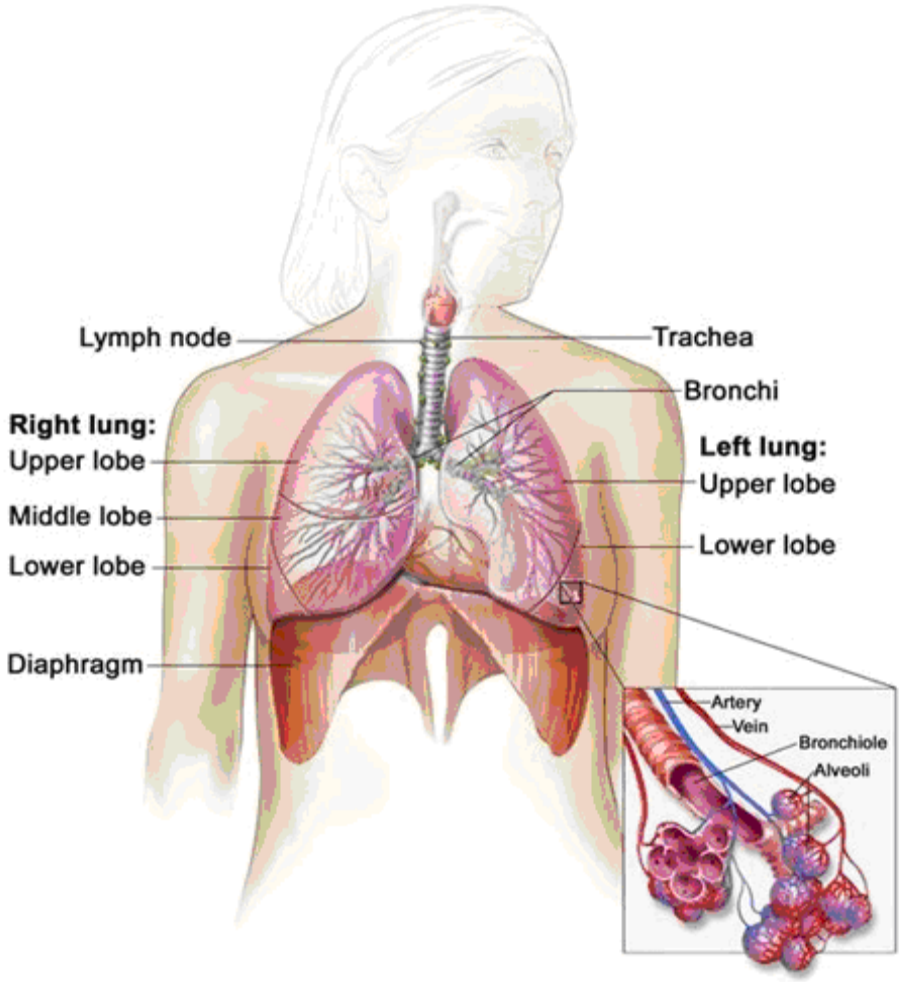

Figure 2.1: Anatomy of the respiratory system, showing the trachea and both lungs, and their lobes and airways (Adapted from the National Cancer Institute).

The primary function of the respiratory system is to supply the blood with oxygen so that this can be delivered to all parts of the body and remove carbon dioxide. The

respiratory system achieves this through the breathing process where oxygen is inhaled and carbon dioxide is exhaled.

The upper respiratory tract comprises of the nose and mouth through which air enters then passing through the larynx and epiglottis. The main function of this region is heating, moistening, and filtering of air. As the atmospheric air has a usual temperature of approximately 20°C and 40-60% moisture, in the upper respiratory tract the air is heated to 37°C and moistened to 99% relative humidity. The lower respiratory tract begins with trachea, a rigid tube that consists primarily of cartilage in the walls. This region of the respiratory tract is referred as the conducting zone and comprises of the first 16 generations of branching. The airways of the conducting zone conduct the oxygen until it reaches the chest cavity where the trachea tube then split symmetrically into two smaller tubes called the bronchi. Each bronchus then divides again forming the bronchioles. This region is the transitional zone and comprises generations 17 through to 19 of branching. The respiratory bronchioles lead directly into the lung where they divide into smaller tubes that connect to tiny alveoli sacs in which gas exchange occurs. This region of the respiratory tract is known as the respiratory zone and comprises of generations 20 to 23 of the branching. Here the inhaled oxygen is delivered into the alveoli and then diffuses through the capillaries into the arterial blood. Meanwhile, the waste from the oxygenated blood is released as carbon dioxide into the alveoli, following the same path out of the lungs through expiration.

The most important part of the respiratory tract in terms of physiological control of the airways is the smaller bronchi and bronchioles. This is because the number of airways doubles at each airway branching normally splitting into two daughters. Therefore, with increasing generation number, the number of branches increases, while the airway diameter at each generation and the distance between branches decrease. The summed

cross sectional area from the mouth to the alveolar sacs increases and results in a trumpet shaped model, with a total absorptive surface area of up to 100 m^2 (Hickey, 1996).

2.2 Asthma

Asthma is a chronic respiratory disease where the respiratory system airways become hypersensitive. Asthma attacks can be triggered by elements such as viral infections, air pollution, tobacco smokes, factory fumes, cleaning solvents, pollens, foods, cold air, and exercise. These triggers constrict and inflame the airways, which reduces the flow of air hence causing difficulties in breathing. Asthma is associated with wheezing, shortness of breath, and a tight feeling in the chest. If the effect on the airways becomes severe enough to impede exhalation results in carbon dioxide build up in the lungs, which may to lead to unconsciousness or death.

Asthma is one of the most common chronic diseases with the highest prevalence in children (Akinbami and Schoendorf 2002). In the United Kingdom over 5.2 million people have asthma, with 1.1 million of these being children (Asthma UK, 2010). Children who develop asthma at a very young age are more likely to grow out of the condition as they get older. Interestingly, asthma can return in the adulthood, especially if during childhood the symptoms of asthma are moderate to severe (Jenkins et al, 1994). Over 70,000 people are admitted to hospital in England with Asthma attacks and there is one death every 7 hours from asthma. Asthma is estimated to cost the United kingdom over 2.3 billion per year (Asthma UK, 2010). Asthma is originally described as an immunological disease where the immune system hyper-reacts to the inhalation of allergens, aggravating inflammation throughout the small and medium airways (Hamid et al, 2003). As a result mast cells bind to IgE to induce de-granulation of the cell and

the release of histamine and other pro-inflammatory mediators including prostaglandins, neutrophils, chemotactic factors, leukotriene, and cytokines causing sudden bronchoconstriction of the airways, which manifest as the immediate asthma response (Figure 2.2).

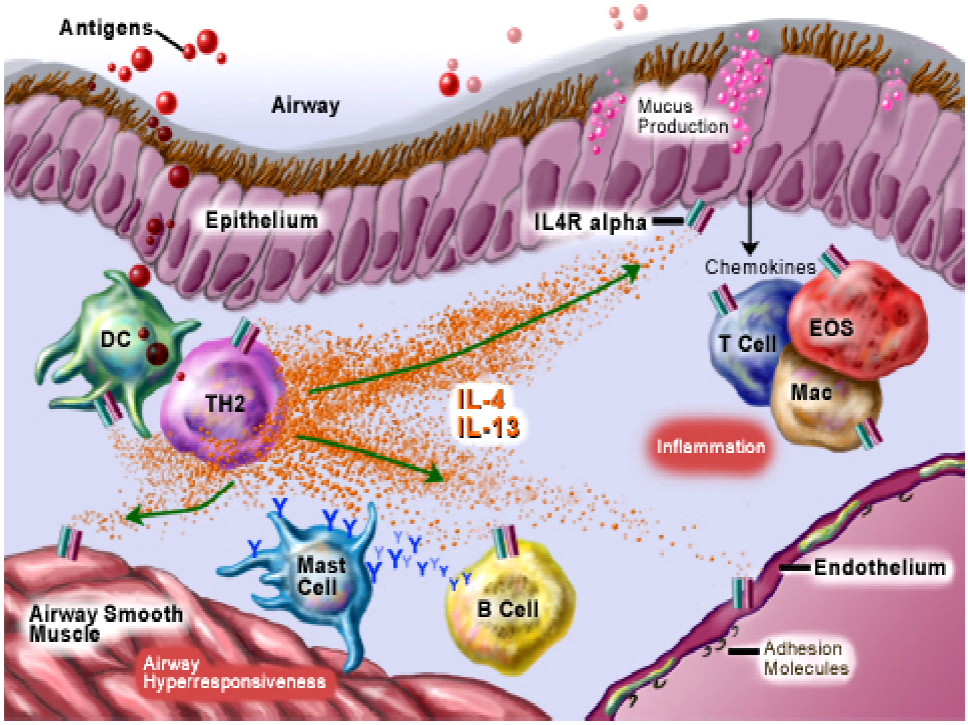

Figure 2.2: Mechanism of asthma (Adapted from Holgate et al, 1996).

Asthma diagnosis requires a detailed medical history and the relevant history of allergic conditions in the family. Additionally the diagnosis is usually confirmed by the peak expiratory flow rate (PEFR) test.

Less commonly, other tests including X-ray are done to eliminate the possibility of other breathing problems. Similarly allergy test or blood test may also be carried out to eliminate the possibility of food allergies.

The treatment of asthma is usually planned by combining medicines and asthma management in the best way to suit the individual. The British Thoracic Society (BTS

2007) has recommended a 5-step approach for the management of asthma as illustrated in figure 2.3.

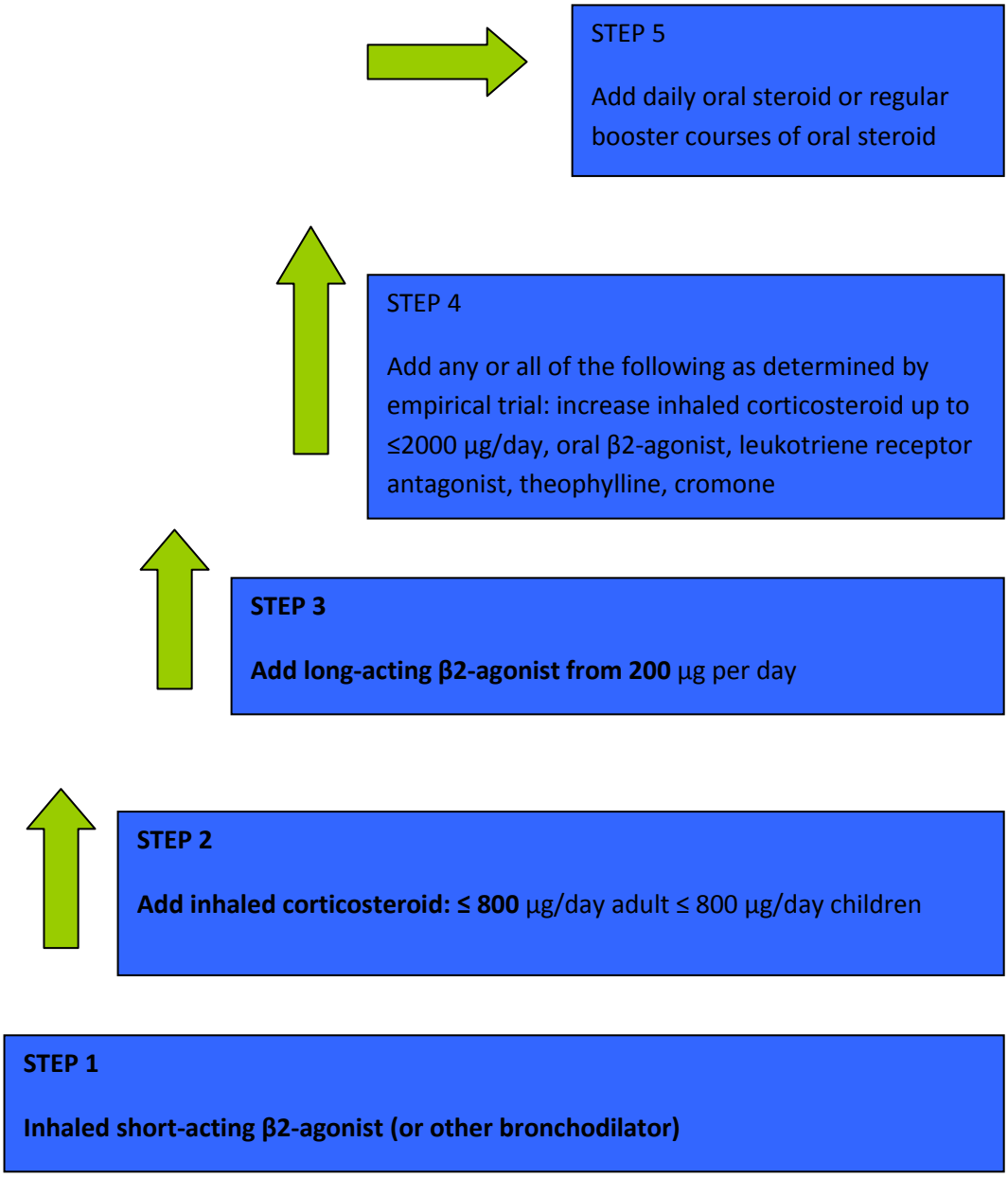

Figure 2.3: BTS/SIGN stepwise approach for the management of asthma (www.brit-thoracic.org.uk)

2.3 Chronic Obstructive Pulmonary Disease (COPD)

Chronic obstructive pulmonary disease (COPD) is the name for the collection of lung diseases including chronic bronchitis and emphysema. COPD is one of the most

common respiratory diseases in the United Kingdom. It usually affects people over 40 years of age.

Around 900,000 people in the United Kingdom have been diagnosed with COPD. However, numbers may be much higher as many people who develop COPD do not seek medical attention because they often dismiss the coughing symptoms as the result of smoking.

A report by the British Lung foundation in 2007 has reported that in the United Kingdom there are 3.7 million people living with COPD. Reports also demonstrated that COPD affects more men than women, but observations suggest that the rate among women is increasing (British Lung Foundation, 2007).

COPD is a disease state characterised by airflow limitation that is not entirely reversible. The airflow limitation is slow and progressive that is usually associated with an abnormal inflammatory response of the lungs to foreign particles and gases. This is primarily originated by smoking which accounts for 90% of all COPD cases (Wetterlin, 1998; National Institute of Health and Clinical Excellence (NICE) 2004; Global Initiative for Chroninc Obstructive Lung Disease (GOLD) 2007). In rare cases, a genetic condition called alpha-1 antitrypsin deficiency can play a role in causing COPD with emphysema occurring before the age of 40 (Laurell and Eriksson, 1963). The inflammatory response due to cigarette smoking and other irritants results in a number of effects such as the activation of macrophages, neutrophils and lymphocytes (Figure 2.4). This results in injury to the airways that with repeated inhalation of irritants cause thickened airways and structural changes including hyperplasia of mucous glands. These processes result in emphysema, chronic bronchitis, or both.

Emphysema is the primary underlying process and is defined by permanent enlargement of airspaces distal to the terminal bronchioles (Figure 2.5). This leads to a dramatic

decline in the alveolar surface area available for gas exchange. Chronic bronchitis leads to obstruction by causing narrowing of both the large and small airways (Figure 2.5). Airways become restricted due to the excessive mucus production and the cilia function weakens. As a result patients face increasing difficulty clearing secretions with disease progression (Soler, 1994).

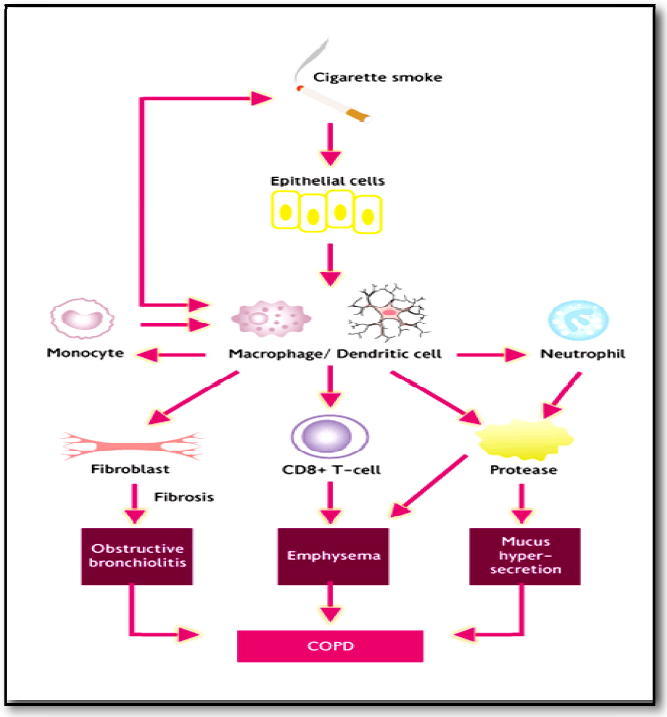

Figure 2.4: Disease process in COPD (Adapted from Barnes et al, 2004).

The diagnosis of COPD is confirmed by spirometry. These measurements also help to determine the severity of COPD, which are classified into mild (FEV_1<80%), moderate (50-70%), severe (30-49%), and very severe (<30%) (Celi, 2004).

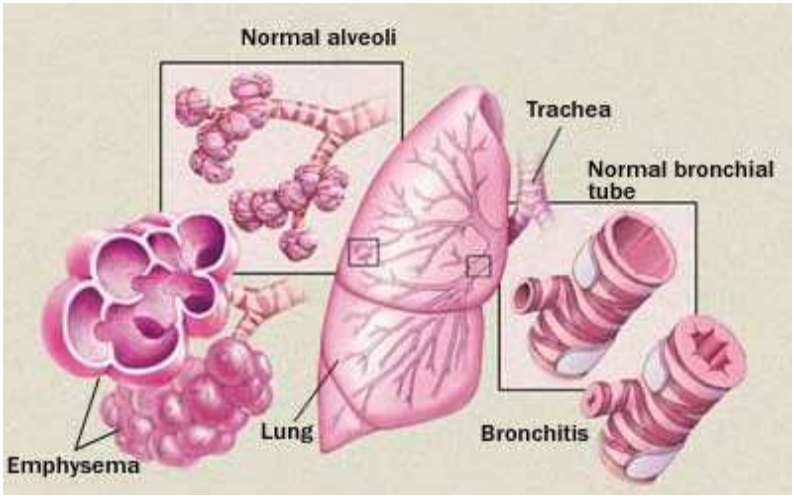

Figure 2.5: Airways of the normal and diseased lungs (Adapted from Mayo Foundation for Medical Education and Research).

The key strategy in the management of COPD is to encourage smoking cessation as this is the most effective way to improve outcomes and prevent further accelerated airway obstruction (Browen et al, 1999).

In retrospective, although asthma and COPD have similar characteristics, they are two distinct conditions in terms of disease onset, frequency of symptoms and reversibility of airway obstruction (Table 2.1).

Table 2.1: Differential diagnosis for COPD and asthma.

	COPD	**Asthma**
Smoker or Ex-smoker	Nearly all	Possibly
Symptoms under age 45	Rare	Often
Chronic productive cough	Common	Uncommon
Breathlessness	Persistent and progressive	Variable

Night time waking with breathlessness and or wheeze	Uncommon	Common
Significant day to day variability of symptoms	Uncommon	Common

2.4 Therapeutic targets

For the inhalation therapy to be efficacious, an adequate dose of the drug must deposit in the lungs. In addition, it is likely that targeting the inhaled mass for deposition in regions of the lung that contain the drug's effecter cells or receptors will also improve efficacy.

Barnes et al (1982) showed that β-receptors located near airway smooth muscle and their numbers increase in smaller airways. The study also showed that large numbers of these receptors are found in the alveoli (Barnes et al, 1982). It is clear that for optimal effects these drugs should be directed to those regions of the lung where the amount of smooth muscle and the populations of these receptors are high.

In asthma and COPD the airways tighten due to inflammation and can be blocked by mucous hence making it difficult for air to get into and out of the lungs. Inhaling medicines allows them to work directly in the lungs where it is needed. Bronchodilation has a clear target in the lungs. The β_2-agonist such as formoterol and salbutamol, relaxes smooth muscle by occupying β_2 receptors, while anticholinergic drugs such as ipratropium bromide, prevent constriction of smooth muscle by occupying cholinergic receptors. Similarly, steroidal and non-steroidal anti-inflammatory drugs such as corticosteroids, sodium cromoglycate, and nedocromil sodium, respectively, are used to treat asthma and COPD by targeting inflammatory

reaction (Faulet al, 1997). The β-agonists have reached a prominent place in the management of asthma and COPD due to their stability and efficacy profile. Formoterol is a selective long-acting β2-agonist bronchodilator with a fast onset of action that when inhaled results in a rapid and long-acting relaxation of the bronchial smooth muscle in patients with reversible airways obstruction (Seborova and Anderson 2001). The long duration of formoterol action occurs because the formoterol molecules initially diffuse into the plasma membrane of the lung cells, and then are slowly released back outside, where they can come into contact with β_2 adrenergic receptors (Arvidsson et al, 1993, Lotvall et al, 1993). Formoterol has been demonstrated to have a faster onset of action compared to other long acting β_2 agonist such as salmeterol due to lower lipophilicity (Linsen et al, 1993). Due to its long duration of action, formoterol is effective in preventing nocturnal asthma symptoms (Vervloet et al, 1998). Formoterol can also be used to prevent occasional exercise-induced bronchospasm (Fernandez and Calix, 2005). The pharmacologic effects of β_2 adrenoceptor agonist drugs, including formoterol, are at least in part attributable to stimulation of intracellular adenyl cyclase, the enzyme that catalyzes the conversion of adenosine triphosphate (ATP) to cyclic-3', 5'-adenosine monophosphate (cyclic AMP). Increased cyclic AMP levels cause relaxation of bronchial smooth muscle and inhibition of release of mediators of immediate hypersensitivity from cells, especially from mast cells (DeBellis and Ronald, 2005). In patients with COPD formoterol have been shown to have a fast onset of effect as rapid as short-acting β-agonist bronchodilator salbutamol (Cazzola et al 2001) and faster than others long-acting β-agonist bronchodilators such as salmeterol (Cote et al 2009) as well as long duration (12 hours). Therefore, formoterol are generally used for maintenance treatment in conjugation with inhaled corticosteroids and due to its rapid onset of bronchodilation it is also given as required or on regular basis, to prevent or reduce symptoms and help the patient perform normal daily activities or improve

exercise tolerance (Bowen et al, 1999 Seborova and Anderson 2000). Moreover studies have shown that as needed formoterol can reduce the evidence of severe asthma exacerbations compared to as needed terbutaline (Tattersfield et al 2001) with lower overall management cost (Berggren and Ekstrem 2001).

2.5 Formeterol Fumarate

Formoterol is a highly selective β_2 adrenergic receptor agonist used in the management of asthma and/or chronic COPD. Its chemical name is (±)-2-hydroxy-5-[(1RS)-1-hydroxy-2-[{(1RS)-2-(4-methoxyphenyl)-1-methylethyl}-aminoethyl] formanilide (Merck index, 1996).

Formoterol fumarate has a molecular weight of 840.9, and its empirical formula is $C_{19}H_{24}N_2O_4)_2 \cdot C_4H_4O_4.2H_2O$ (Figure 2.18). It is a phenylethylamine derivative with one phenolic hydroxyl and one secondary amino group, and is widely marketed as a racemate of the enantiomers, which have the RR+SS configuration (Cherkaoui et al, 1999). Formoterol fumarate is a white to yellowish crystalline powder, which is freely soluble in glacial acetic acid, soluble in methanol, meanly soluble in ethanol and isopropanol, slightly soluble in water, and practically insoluble in acetone, ethyl acetate, and diethyl ether (Merck Index, 1996).

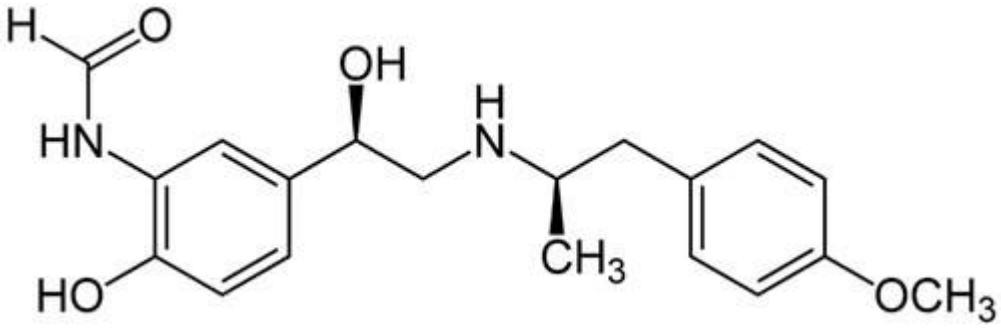

Figure 2.6: Molecular structure of formoterol.

2.5.1 Pharmacokinetics and pharmacodynamics of formoterol

2.5.1.1 Pharmacokinetics

Information on the pharmacokinetics of formoterol in plasma has been obtained in safety studies involving healthy subjects by oral inhalation of doses higher than the recommended range and also in COPD patients after oral inhalation of doses at and above the therapeutic dose (Lofdahl and Svedmyr, 1989). Urinary excretion of unchanged formoterol has been used as an indirect measure of systemic exposure (Chuchalin et al, 2005). Plasma drug disposition data parallel urinary excretion, and the elimination half-lives calculated for urine and plasma are similar.

2.5.1.2 Absorption

As with other substances administered by inhalation, 90% of the inhaled formoterol dose is swallowed and absorbed from the gastrointestinal tract (Barnes, 2010). Following inhalation of a single 120 μg dose of formoterol fumarate by 12 healthy subjects, formoterol was rapidly absorbed into the plasma, reaching a maximum drug concentration of 92pg/mL within 5 minutes of dosing. In COPD patients treated for 12 weeks with formoterol fumarate 12 or 24 μg., the mean plasma concentrations of

formoterol ranged between 4.0 and 8.8 pg/mL and 8.0 and 17.3 pg/mL, respectively, at 10 min, 2 h and 6 h post inhalation (Campestrini et al, 1997). Following inhalation of 12 to 96 μg of formoterol fumarate by 10 healthy males, urinary excretion of both (R,R) - and (S,S)-enantiomers of formoterol increased proportionally to the dose. Thus, absorption of formoterol following inhalation appeared linear over the dose range studied (Rosenborg et al, 1999). In a study in patients with asthma, when formoterol 12 or 24 μg twice daily was given by oral inhalation for 4 weeks or 12 weeks, the accumulation index, based on the urinary excretion of unchanged formoterol ranged from 1.63 to 2.08 in comparison with the first dose. For COPD patients, when formoterol 12 or 24 μg twice daily was given by oral inhalation for 12 weeks, the accumulation index, based on the urinary excretion of unchanged formoterol was 1.19 - 1.38 (Campestrini et al, 1997). This suggests accumulation of formoterol in plasma with multiple dosing. The excreted amounts of formoterol at steady-state were close to those predicted based on single-dose kinetics. As with many drug products for oral inhalation, it is likely that the majority of the inhaled formoterol fumarate delivered is swallowed and then absorbed from the gastrointestinal tract. The pharmacokinetics of formoterol has not been studied in the elderly population, and limited data are available in pediatric patients.

2.5.1.3 Distribution

The binding of formoterol to human plasma proteins in vitro was 61%-64% at concentrations from 0.1 to 100 ng/mL (Novartis Pharma AG, 2003). Binding to human serum albumin in vitro was 31%-38% over a range of 5 to 500 ng/mL (Novartis Pharma AG, 2003). The concentrations of formoterol used to assess the plasma protein binding were higher than those achieved in plasma following inhalation of a single 120 μg dose.

2.5.1.4 Metabolism

Formoterol is metabolised primarily by direct glucuronidation at either the phenolic or aliphatic hydroxyl group and O-demethylation followed by glucuronide conjugation at either phenolic hydroxyl groups (Boulton and Fawcett, 2002). Minor pathways involve sulfate conjugation of formoterol and deformylation followed by sulfate conjugation. The most prominent pathway involves direct conjugation at the phenolic hydroxyl group (Boulton and Fawcett, 2002). The second major pathway involves O-demethylation followed by conjugation at the phenolic 2'hydroxyl group. Four cytochrome P450 isozymes (CYP2D6, CYP2C19, CYP2C9 and CYP2A6) are involved in the O-demethylation of formoterol. Formoterol did not inhibit CYP450 enzymes at therapeutically relevant concentrations. Some patients may be deficient in CYP2D6 or 2C19 or both.

2.5.1.5 Excretion

Renal clearance of formoterol from blood in these subjects was about 150 mL/min. Following inhalation of a 12 μg or 24 μg dose by 16 patients with asthma, about 10% and 15%-18% (Novartis Pharma AG, 2003) of the total dose was excreted in the urine as unchanged formoterol and direct conjugates of formoterol, respectively. Following inhalation of 12 μg or 24 μg dose by 18 patients with COPD the corresponding values were 7% and 6-9% of the dose, respectively.

2.5.1.6 Side effects

The major adverse effects of inhaled beta$_2$-agonists occur as a result of excessive activation of the systemic β-adrenergic receptors. The most common adverse effects in adults and adolescents include skeletal muscle tremor and cramps, insomnia, tachycardia, decreases in plasma potassium, and increases in plasma glucose.

The relationships between heart rate, ECG parameters, and serum potassium levels and the urinary excretion of formoterol were evaluated in 10 healthy male volunteers (25 to 45 years of age) following inhalation of single doses containing 12, 24, 48, or 96 μg of formoterol fumarate (Rosenborg et al, 1999). There was a linear relationship between urinary formoterol excretion and decreases in serum potassium, increases in plasma glucose, and increases in heart rate. In separate studies the relationships between plasma formoterol levels and pulse rate, ECG parameters, and plasma potassium levels were evaluated in 12 healthy volunteers following inhalation of a single 120 μg dose of formoterol fumarate (10 times the recommended clinical dose). Reductions of plasma potassium concentration were observed in all subjects (Lecaillon et al, 1999). Maximum reductions from baseline ranged from 0.55 to 1.52 mmol/L with a median maximum reduction of 1.01 mmol/L. The formoterol plasma concentration was highly correlated with the reduction in plasma potassium concentration. Generally, the maximum effect on plasma potassium was noted 1 to 3 hours after peak formoterol plasma concentrations were achieved.

2.5.2 In vivo and in vitro methods for the determination of formoterol

Formoterol has two asymmetric centres but it is used clinically as the racemic mixture of the active (R, R) and inactive (S, S) formoterol (Trofast et al, 1991). Determination of formoterol in biological fluids has been challenging due to low system concentrations hence limiting detection. However, there are literatures suggesting that hyphenated techniques coupling HPLC with mass spectophotometer (Marzo et al, 2000) and gas chromatography with mass spectrophotometer (Damasceno et al, 2002) are used as detection methods. Additionally, an ultra –sensitive method has been reported for the determination of formoterol in human serum using liquid chromatography/mass

spectophotometry with an electrospray interface. This method produced a limit of quantification as low as 0.40 pg/mL with a precision of 19.67% and an accuracy of 96.78% (Mascher et al, 2006).

As a consequence of low inhalation dosages, the amount of formoterol which lead to plasma concentrations is in the low picograms per millimeter range. Hence the evaluation of pharmacokinetics at therapeutic doses has primarily been focused on urine excretion (Kamimura et al, 1982, Nadarassan et al, 2007). The choice of method to be used is dependent on factors such as cost, skill of the analyst, facilities available, number of specimens expected, and the time available in which to produce the results of the study (Wenk, 1982). For instance, satisfactory agreement was obtained for levels of formoterol in plasma and urine when they were determined by radioimmunoassay and gas chromatography-mass spectrometry. The concentration of formoterol determined in human urine after oral administration of formoterol fumarate was 40 μg/L (Yokoi et al, 1983). Capillary electrophoresis equipped with laser is also suitable for the analysis of racemic formoterol and formoterol enentiomers (Cherkaoui et al, 1995). The proposed method is reliable, precise, accurate, fast, and cost effective. Other studies have been reported on an automated HPCL method for the determination of formoterol in human plasma using electrochemical detection technique. The results showed a limit of quantification of 7.14 pmol/l of formoterol base and 3 pg/ml of formoterol fumarate dehydrate with accuracy over the entire concentration range varying from 98 to 109% and precision ranging from 8 to 19%. Although the analysis claims to have high sensitivity and selectivity the electrochemical detection technique has low reproducibility (Campestrini et al, 1997). In separate studies, a novel HPLC method for the estimation of formoterol in urine samples was developed and validated by Nadarassan et al (2007). The limit of quantification for formoterol was found to be 1.50

ng/mL, with an accuracy of 95% and a precision of around 3.7% (Nadarassan et al, 2007).

2.5.3 Physical and chemical properties of formoterol

The chemical and physical properties of formoterol are of great importance in drug formulation as it is essential for the generation of fine particles, in the ideal particle size range, whilst maintaining aerodynamic properties required inhalation.

Inhaled medication usually comprises very small amounts of drugs that have to be accurately metered (6 μm to 500 μm) and special powder formulations are necessary to obtain free flowing powder that can be used for processing and metering.

For instance, DPI powder formulation requires the addition of a second or third agent after production to improve powder flow, dose reproducibility, and act as a diluent for highly active compounds to fulfill regulatory requirements and produce the anticipated clinical outcomes.

For the various DPI currently available in the market, only two different types of powder formulations are currently used. Spherical pellets are one type of formulation where the micronised drug particles are agglomerated into much larger spherical units without a binding agent, behaving as a free flowing powder. Spherical pellets can disintegrate nearly completely during inhalation into much smaller agglomerates or even primary particles that have the required size-range for deep penetration into the respiratory tract. Spherical pellets are used in the Turbuhaler®.

The other type of formulation consists of relatively large carrier crystals, mostly α-lactose monohydrate, carrying the micronised drug particles which are distributed over their surface. During inhalation, the drug particles have to be detached from the carrier

crystals to generate the aerosol with particles of the desired particle size, which are able to enter the lower respiratory tract. The α-lactose monohydrate is used in the Aeroliser® containing 12μg and 25 mg lactose as a single dose delivery system.

For the MDI, formoterol has proved difficult to formulate due to both physical and chemical stability challenges including relatively low dose requirements (12μg) and chemical instability, which can lead to a decrease in formoterol content on storage, allowing only a limited shelf life.

Formoterol has previously been marketed as a Foradil® Inhaler. This was a chlorofluorocarbon

(CFC) based MDI solution formulation and provided a shelf life of 12 months refrigerated storage prior to use, followed by a 3 month ambient in use period.

With the phase out of CFC and their replacement with HFA (hydrofluoroalkane), an opportunity arose to reformulate and improve on the performance of the older CFC products.

Currently the only formoterol HFA-based MDI products available on the European market are 12μg per actuation solution inhalers marketed as Atimos®.

There are two types of HFA MDI formulation, solutions and suspensions. Drugs formulated as solutions have the advantage of being fully homogenous and are likely to provide the best dosing uniformity. However, chemical degradation is more likely to occur with a solution system and this is evident with the solution formulation systems of formoterol (Bainbridge and Jinks,). Suspension formulations on the other hand are less prone to drug degradation but can exhibit inconsistencies in dosing behaviour (Jinks, 2003) that are especially apparent with low dose products such as formoterol.

2.6 Inhaler Devices containing formoterol

The lung has served as a route of drug administration for thousands of years. The origin of inhaled therapies can be traced back 4000 years ago to India, where people smoked the leaves of the *Atropa belladonna* plant to suppress cough (Labiris and Dolovich, 2003). In the ninetieth and early twentieth centuries, asthmatics smoked asthma cigarettes that contained stramonium powder mixed with tobacco to treat the symptoms of their disease (Labiris and Dolovich, 2003). The development of modern inhalation devices has subsequently seen the refinement of the nebuliser and the evolution of two types of compact portable devices, the MDI and DPI (Labiris and Dolovich, 2003).

Inhaled formoterol fumarate products are marketed as Oxis® Turbuhaler® (Astra Zeneca) and the Foradil® Aeroliser® (Norvatis Pharma) as DPI and as Atimos® Modulite® (Trinity-Chiese) in MDI.

The Oxis® Turbuhaler® is a multi dose reservoir system (Table 2.2). In this type of inhaler, the powder formulation is stored in a hopper from which single doses are measured volumetrically with an especial dose-metering unit. Each metered dose contains either 6 μg or 12 μg of formoterol fumarate.

Multi-dose reservoir devices contain more than one dose of the drug. There are two types of multi-dose DPI devices, reservoir and multi-unit dose devices. Multi-dose reservoir devices contain bulk supply of the drug from which individual doses are released from each actuation. The first of multi-dose reservoir devices to be developed was the Turbuhaler®. The drug located within this inhaler is formulated as a pellet of a soft aggregate of micronised drug which may be formulated with or without any additional lactose excipient. To release a dose of the drug, the patient twists the base of the device resulting in the dose of the drug being shaved off the pellet. Upon inhalation,

the dose is then dispersed by turbulent airflow, which creates the energy to disperse particles in the emitted dose.

The Foradil® Aeroliser® is a single dose inhaler which consists of a capsule dosage form containing a dry powder formulation of formoterol fumarate intended for oral inhalation only with an Aeroliser® inhaler (Table 2.2). Each capsule contains a dry powder blend of 12µ formoterol fumarate and 25mg of lactose as a carrier.

In the single-unit devices the drug that is formulated as a micronised powder in a lactose excipient, is supplied in individual single-dose gelatin capsules, which must be inserted into the inhaler before use.

The Atimos® Modulite® MDI delivers a metered dose of 6µg and 12µg formoterol fumarate from a CFC-free propellant metered dose inhaler using modulite formulation technology (Ganderton et al, 2002).

The MDI is a device that delivers a specific amount of medication to the lungs in the form of a short burst of aerosolised medicine that is inhaled by the patient. The medication in a metered dose inhaler is most commonly a bronchodilator, a corticosteroid, or a combination of both for the treatment of asthma and COPD.

In line with this, combination products are also available. These include Fostair inhaler (Trinity-Chiese), which is the first and only CFC-free MDI combining active ingredients, beclometasone dipropionate (cortocosteroids) 100 µg and formoterol fumarate 6 µg.

Table 2.2: Dry powder inhalers available in the market (Adapted from Chrystyn, 2007).

Inhaler Device (manufacturer)
Single dose inhalers
Spinhaler® (Rhone-Poulenc Rorer)
Cyclohaler® (Pharmachemie)
Rotahaler® (Glaxo Smithkline)
Spinhaler® (Aventis)
Aeroliser®- Foradil DPI (Norvatis Pharma)
Spiriva Handihaler® (Boehringer Ingelheim)
Inhalator® (Boehringer Ingelheim)
Multi-dose inhalers
Multi unit-dose inhalers
Aerohaler® (Boehringer Ingelheim)
Diskhaler® (Glaxosmithkline)
Diskus/Accuhaler® (Glaxosmithkline)
Multi-dose Reservoir systems
Clickhaler® (Innovata Biomed/ML Labs Celltechn)
Easyhaler® (Orion Pharma)
Pulvinal® (Chiesi)
Turbuhaler® (AstraZeneca)
Twisthaler® ((Schering-Plough)
Novoliser® (ASTA Medica)

2.7 Particles

2.7.1 Particles aerodynamic in the Respiratory Tract

The success of inhaled therapy depends on the ability to deliver adequate aerolised drug in the optimal size range to appropriate sites in the lungs with minimal side effects. In comparison to other forms of therapy, inhaled therapy provide ease of administration, topical delivery of minute but effective doses to therapeutic active sites, rapid action and avoidance of gastrointestinal upset from oral therapy (Barnes, 2004, Chrystyn, 2006).

To achieve an effective drug delivery system to the lungs several factors including particle, size inspiratory flow rate, lung volume at the time of inhalation and breath-holding time must be well understood.

The particle size of an inhalation aerosol is important, because only the particles that are fine enough can gain access to the airways and be available for therapeutic benefit in the lungs (Dolovich and Newhouse, 1993).

Particle size is defined by several parameters including mass median aerodynamic diameter (MMAD) of the aerosol, which refers to the cumulative distribution at the 50% point of the aerosol mass diameter and geometric standard deviation (GSD), which is a measure of the variability of the particle diameters within the aerosol (Labiris and Dolovich, 2003).

Both the MMAD and GSD give a measure of heterogeneity of the aerosol and serves as predictors for the site of deposition in the lungs as well as indicating the dose or collective amount of the drug carried by the aerosol.

In addition to MMAD and GSD, the fine particle fraction (FPF), or percentage of particles within the aerosol that are less than 5 μm in diameter (Newman, 1991), is also used to describe the quality of an aerosol and potential for targeting and delivering

sufficient amounts of the drug to the lungs. Therefore, the percentage of particles with increasing likelihood for depositing in the distal lungs increases as the FPF increases, also indicating that the aerosol has a smaller MMAD.

The drug–device combinations must aerosolise the drug into the appropriate particle size distribution and concentration to ensure optimal dose and deposition in the desired region of the lung with minimal side effects.

2.7.2 Particle Deposition

Particle deposition is governed by the local dynamics of aerosol particles that are carried through the complex geometry of the airways by the inspiratory and expiratory airflow. This occurs as a result of three basic mechanisms: inertial impaction, gravitational sedimentation, and Brownian motion (Lippman et al, 1980).

Airborne particles travelling through the respiratory tract are subject to constantly changing forces as a result of different bends and a decreasing air velocity. In the relatively wide upper airways, where the air velocity is the highest, inertial force is dominant. Particles enter the airway system with air velocity originated through inspiration force applied to the inhaler. Aerosol particles have to follow the streamlines of the air in bends and burifications in order to penetrate deeper, but are unable to perform this when the inertia is too high. Therefore the larger particles are deposited by the mechanism of inertial sedimentation in the throat and burifications of the larger airways. As the remaining small particles move further down the airways, the air velocity gradually decreases to much lower values. In these smaller airways, air flow is slow and particle deposition due to this force of gravity becomes important. Thus, the particles settle by sedimentation in these regions of the respiratory tract. However, the settling velocity is too low and residence time can be too short to remove the smallest

particles in the aerosol cloud from the air by this mechanism. So these finest fractions move by Brownian motion and are able to make contact with the walls of the airways (Figure 2.7).

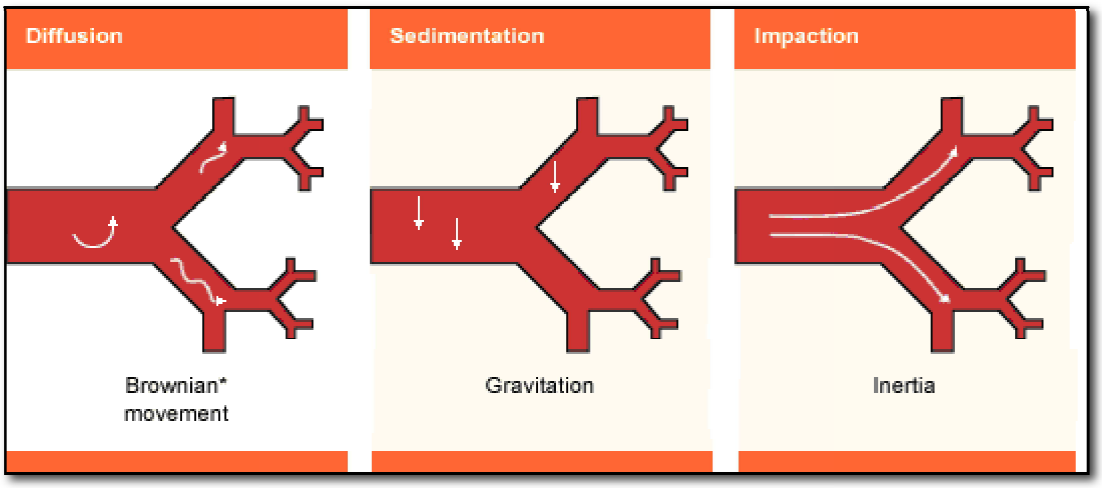

Figure 2.7: Particle deposition mechanisms at airway branching site (Adapted from Voshaar, 2005).

Particles entering the respiratory tract do not only vary in size and velocity, but also in shape and density, depending upon the type of drug and the inhalation system used for aerosol generation. As a result the aerodynamic particle diameter was introduced to compare the behaviour of different type of aerosol particles.

Deposition efficiency for particles in the respiratory tract is therefore presented as a function of their aerodynamic diameter (Patton, 1996). Large particles (>8 µm) are predominately deposited in the oropharyngeal region (Gerity, 1990). Particles with a size between 1-5 µm in diameter are deposited in the small airways and alveoli with approximately 50% of 3 µm diameter particles being deposited in the alveolar region (Effros and Mason, 1983). Smaller particles <3 µm have an approximately 80% chance

of reaching the lower airways with 50-60% being deposited in the alveoli (Patton, 1996) (Figure 2.8).This mechanism increases inversely proportional with particle size and directly proportional with length of stay in the lungs.

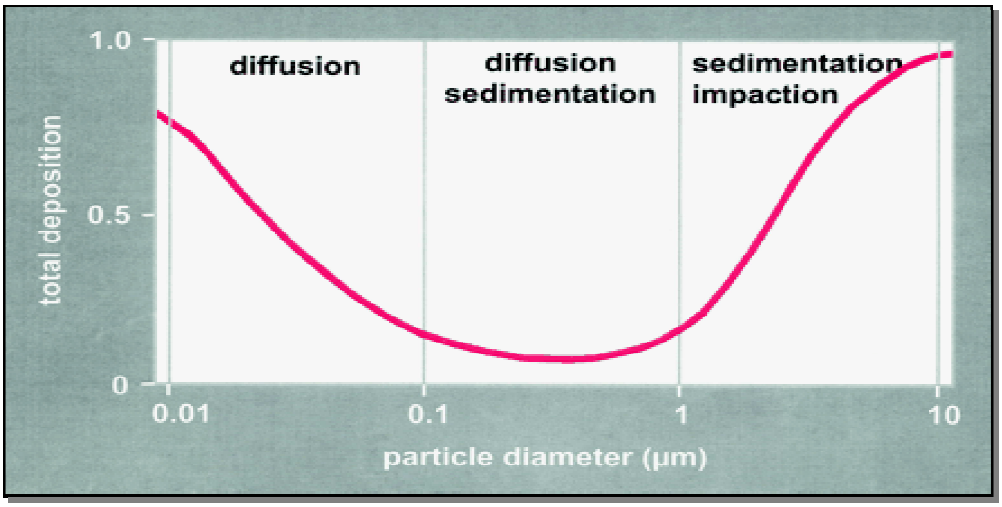

Figure 2.8: Total deposition of particles in human respiratory tract inhaled orally (Heyder, 2004).

2.8 Methods of studying drug deposition

The size classification of the aerosol in terms of its aerodynamic behaviour of the inhaled products can be performed by different methods including in vitro dose emission and particle size distribution measurements, in vivo gamma scintigraphy (radioaerosol drug deposition), pharmacokinetic, and pharmacodynamics. These methods used individually or in combination can be used to determine the fate of the inhaled drug particles as the pulmonary fate of the aerolised drug is influenced by where the aerosol particle is deposited in the lung (Chrystyn, 2000, Chrystyn, 2001).

2.8.1 In vitro methods

Important parameters for inhalation product performance are the amount of drug delivered to the patient and the effective aerodynamic particle size of the drug particles. To estimate the amount of the drug actually available to deposit in the patient's lungs a combination of the total emitted dose and the fine particle dose or the mass of particles with an aerodynamic diameter between 1 and 5μm that have the highest probability of depositing in the lung can be useful.

In the case of a DPI both the emitted and fine particle dose is affected by the strength and duration of the patient's inspiration (Newman et al, 1991; Ross and Schultz, 1996). Furthermore, different inhalers provide varying degrees of resistance to flow (Clark and Hollingworth, 1993). Moreover, particularly when testing DPIs of intermediate to high flow resistance, it is essential to determine the appropriate test flow and duration based on the pressure drop developed over the specific inhaler under test.

In line with this, although in vitro test methods were developed by pharmaceutical manufacturers as early as the 1960s, and such methods obviously gathered the approval of regulatory agencies, there were initially no publicly available testing methods and specifications for DPIs (Byron, 1994). To remedy this, the United States Pharmacopeia (USP) developed a standardised method and suggested testing DPI at a constant pressure drop (4.0 kPa), resulting in a specific test flow for the inhaler. In addition, controls were introduced to ensure that appropriate flow rates were instantaneously achieved through DPIs by diverting air through the inhaler using a solenoid valve and an electronic timer. This ensured that the flow through the dose emission unit is set as required and that is unaffected by minor fluctuations in the pump. The resulting airflow that produces a drop of 4.0 kPa over the inhaler to be tested, should be used for the determination of the delivered dose and particle size distributions as recommended by

the compendial methods (European Pharmacopeia, 2007; British Pharmacopeia, 2008; United States Pharmacopeia, 2009)

The only exception to this approach is for low resistance DPI that produce inhalation flow rates above 100 L/min at 4.0 kPa. In this case, a flow of 100L/min should be used.

Current in vitro testing therefore replicates both the peak rate at which a patient might be expected to inhale through a DPI and the patient's volume of inhalation.

Cascade impaction is an in vitro technique often used to determine the size and distribution of inhaled aerosols (Hess et al, 1996). The theory of impactors has been well developed over the years (Marple, 1970). Inertial impactors size-separate particles subjected to a change in flow direction of their support gas, usually air moving at constant flow rate under laminar flow conditions (Marple, 1974). Particles entering a single-stage impactor pass through a plate containing 1 or more jets of well-defined size. A collection surface located immediately beyond the plate at a well-defined separation distance deflects the flow. The inertia of the particles causes them to cross the flow stream, with the result that those with a size greater than a critical value impact on the surface, whilst smaller particles remain airborne. The size at which a given impaction stage collects 50% of the mass entering is termed the effective cutoff diameter and defines the calibration for that stage (Mitchell , 2003). Several stages are arranged in sequence in a cascade impactor, such that particles having progressively finer sizes are collected as the aerosol passes through the instrument.

a) Twin stage impinger

The Twin Stage Impinger Apparatus was the first impactor that was recognised to make measurements of the emitted dose from the inhaler. It was used in product development,

batch release and in applications with add-on devices (Labiris and Dolovich, 2003; Leach, et al., 2002). This apparatus comprises two stage reservoirs (figure 2.9) - stage I, representing the amount of dose deposited in the oropharyngeal region (6> μm) and stage II representing the amount of drug deposited in lungs (6< μm) (Feddah, et al., 2000). The twin impinger apparatus was replaced by more modern methods, as although it was useful for the determination of the fine particle fraction, it provided insufficient rapid resolution in the critical aerodynamic range of 0.5 to 5.0 μm (Newman, 1994).

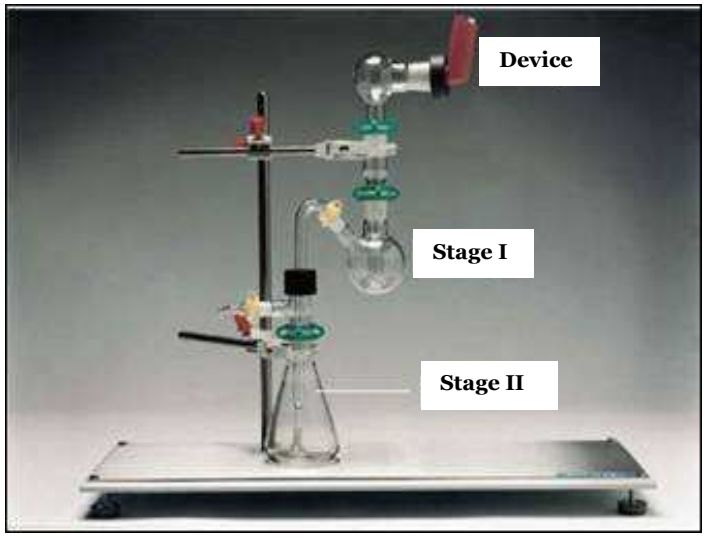

Figure 2.9: The twin stage impinger (Adapted from Copley Scientific, 2006).

b) Multi-Stage Liquid Impinger

The multi-stage Liquid impinger consists of the metal throat, impaction stages and a final filter (Figure 2.10). The apparatus operates at 60L/min and has a cut-off diameter of 25, 13, 6.8, 3.1, 1.7 μm, thus giving a much more detailed particle size distribution than the Twin stage impinger.

Figure 2.10: Multi-stage liquid impinger (Adapted from Copley, 2006).

c) Anderson Cascade Impactor (ACI)

The ACI shown in Figure 2.11, is the most commonly used in vitro method to indentify the aerodynamic characteristics of emitted dose. The apparatus consists of a stack of eight plates, each containing a series of precision drilled holes and a final filter stage (European Pharmacopeia, 2001; British Pharmacopeia, 2005, United States Pharmacopeia, 2005). Its design allows particles of diminishing size to travel through the impactor and to be captured on a series of plates. Each plate has successively smaller exit holes (Clark, 1995).

The standard ACI is designated to operate at a flow of 28.3 L/min with stage cut-off diameters of 9, 5.8, 4.7, 3.3, 2.1, 1.1, 0.7, and 0.4 μm, respectively. This method allows a more descriptive analysis of the particle size distribution than the multi-stage liquid impinger and liquid impinger. Although the ACI is designated to be used at inhalation flows of 28.3 L/min, modifications are available for the use of high flows namely 60 and 90 L/min. For an inhalation flow of 60 L/min stages 0 and 7 are removed and replaced by stages -1 and -0 on the top of the ACI. For an inhalation flow of 90 L/min

stages, 0, 6, and 7 are removed and replaced by stages -2, -1 and -0 on the top of the ACI. In the case of DPIs, a pre-requisite prior to testing is the use a pre-separator with a small amount of solvent being coated on the collection surfaces of the ACI to prevent those particles greater than 10 μm from bouncing into the ACI stages (Figure 2.11).

Moreover, the ability to quantify the amount of drug carried by the aerosol particles of a specific size is useful for interpreting the resulting lung deposition patterns and clinical effects of the inhaled dose.

Therefore, the accuracy in determining these in-vitro doses is increased when using ACI containing modification kits together with the mixing inlet (Copley, 2003). This allows the determination of dose emission and aerodynamic particle size distribution of dose from DPIs and different inhalation flow rates and for different inhaled volumes, hence attempting to match the optimal performance of the inhaler and the patient's actuation (Hindle, 1995).

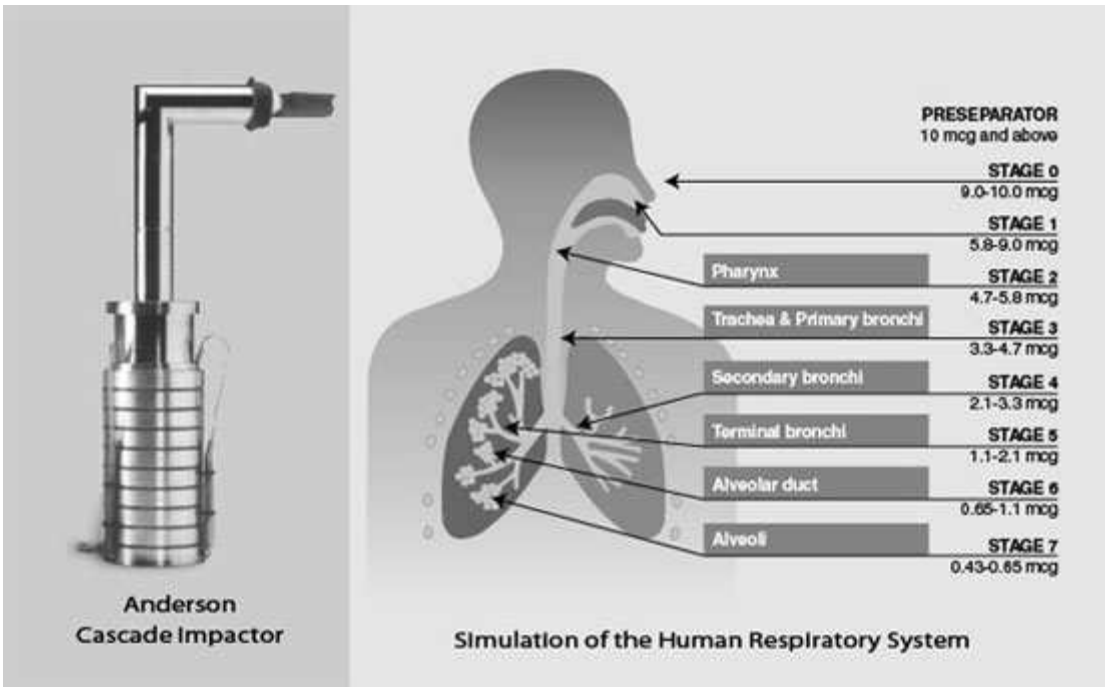

Figure 2.11: An Anderson Cascade Impactor, which is used to test the particle size distribution of aerosols in vitro.

d) Next Generation Impactors (NGI)

The recently introduced Next Generation Pharmaceutical Impactor was designed with the intent of offering a quicker method to the Pharmaceutical industry compared to the Andersen Stage Cascade Impactor.

The NGI is a high performance cascade impactor for classifying aerosol particle into size fractions for testing MDI, DPI and other inhaled drug delivery devices such as nebulizers and nasal sprays (Figure 2.12). This apparatus is recognized internationally both in the USP and the EP. It features removable collection cups, O-ring-free connections, and a micro-orifice collector that can be used in place of a final filter for most formulation. These features make this apparatus very efficient, enabling 10 to 20 inhaler determinations per day with one impactor.

This impactor has a seven impaction stages plus a final micro-orifice collector that can be used in place of a final filter for most formulations. The cut-off aerodynamic diameter ranges from 0.54 μm to 11.7 μm at 30 L/min and 0.24 μm to 6.12 μm at 100 L/min.

The NGI has several features that enhance its utility for inhaler testing. Primarily, the particles deposition on collection cups are held in a tray from the impactor as a single unit, facilitating quick sample turn-around times if multiple trays are used.

Secondly, the user can add up approximately 40 ml of an appropriate solvent directly to the cups for more efficient drug recovery. Thirdly, the micro-orifices collector captures extremely small particles normally collected on the final filter in other impactors. The particles captured in the micro-orifices collector cup can be analysed in the same manner as the particles collected in the other impactor stage cups. The work-effective features and aerodynamic design principles together provide an impactor well suited to the needs of the inhaler testing researchers.

Figure 2.12: Next Generation Impactor (Adapted from MSP, St Paul, MN, 2008)

2.8.2 In vivo methods

2.8.2.1 Pharmacokinetics

When assessing inhaled drug absorption, standard pharmacokinetic analyses cannot differentiate between the drug that reaches the systemic circulation from the lungs and that delivered by the gastrointestinal route.

Pharmacokinetic methods are indirect measurements because they utilise measurements from blood/serum (Seale et al, 1998) or urine (Hindle and Chrystyn, 1992) to identify the relative amounts of drug delivered to the systemic circulation via the lungs and gastrointestinal tract. Thus procedures are required to differentiate between these two routes of absorption (circulatory and gastrointestinal). Pulmonary absorption kinetics can be studied by blocking gastrointestinal absorption with charcoal, or by studying absorption during the first 30 minutes post inhalation before appreciable oral absorption has occurred (log time for absorption).

2.8.2.1.1 Pharmacokinetic studies using blood samples

Pharmacokinetic methods using blood samples have been used to compare lung deposition of inhaled products (salbutamol 200μm) via differently actuated inhalers MDI and DPIs (Diskhaler® and Acchulaher®) (Lipworth and Clark, 1997). The study was carried out using 10 volunteers over 6 minutes and plasma concentrations were measured at 5, 10, 15, 20 minutes post inhalation. Results showed maximum concentrations of 4.35 ng/ml (Diskhaler), 3.98 ng/ml (MDI) and 3.22 ng/ml (Accuhaler®). Such studies provided evidence that both the Dishaler® and MDI produce significantly ($p<0.05$) higher plasma concentration than the Accuhaler®. Pharmacokinetic methodology was also used to assess the pulmonary deposition of budesonide from a Turbuhaler® 200 μg/dose and a fluticasone from a Diskus® 250 μg/dose in asthmatic children with an average age of 12 years. This study was reported to be the first direct in vivo comparison of the lung deposition and pharmacokinetic parameters of these two most widely used DPI devices in children with asthma and also the first to carefully validate simultaneous dosing techniques with different inhaled drugs. The Turbuhaler® was found to deliver almost four times as much drug to the lungs compared to Diskus® at a standard flow rate of 60L/min (Luyt, 1995). Interestingly, a three to four-fold difference in lung deposition mirrors the difference between the fine particle doses of the two inhalers (Martin et al, 2002). This supports the notion that fine particle dose may be a good predictor of lung deposition as suggested in other studies with DPI (Olsson et al, 1996).

2.8.2.1.2 Urinary pharmacokinetic methods

a) Excretion with charcoal block

A charcoal block method with serial urine sampling has also been used to identify the total lung dose. Lung deposition is determined from the urinary excretion of intact drug, provided that the contribution of orally deposited and subsequently swallowed drug can be accounted for, and that the drug is not metabolised in the lung.

This method was first proposed by Borgström & Nilsson (1990). Eleven healthy subjects inhaled four doses of 250 μg terbutaline from a p-MDI with concurrent oral administration of 50 g charcoal suspended in 500 ml of water. The average amount of terbutaline excreted in the urine up to 48 h post-inhalation was 9.1% of the nominal dose. Moreover, following concurrent oral administration of terbutaline and charcoal (50 g over 4 h) in five of the individuals, the average amount of terbutaline excreted in the urine was 0.30% of the nominal dose. Similarly, in studies assessing the total effective dose terbutaline from Turbuhaler® inhaled by six volunteers, reported an average lung deposition of 21.1 % of the nominal dose (Borgström et al, 1992).

A charcoal block method has also been described in 24 healthy subjects after inhalation of budesonide from Turbuhaler® and p-MDI in an open, randomised crossover study. Following budesonide administrations without charcoal, the mouth was immediately rinsed with 200 ml of water, which was then swallowed. When administration with concomitant charcoal was performed, the mouth was thoroughly rinsed of charcoal suspension, which was swallowed immediately and repeated at 5 min, 1 and 2 h after drug administration. The subjects were trained to breathe out to residual volume and then to inhale at a flow of 60L/min for the Turbuhaler® and 30 L/min for the p-MDI. The result demonstrated that the oral availability of budesonide after concomitant charcoal administration was found to be 2.5%. Furthermore, with an oral availability of

13% without charcoal, the preventive effect of charcoal on budesonide absorption can be estimated to be approximately 80%. The pulmonary availability, calculated using the metered doses from Turbuhaler®, with concomitant administration of charcoal, and compensated for the contribution of orally absorbed drug, was 32% for Turbuhaler® and 18% for the P-MDI. The pulmonary availability for Turbuhaler® was about twice that for the p-MDI. The systemic availability of the metered dose of budesonide after inhalation via Turbuhaler® is about 50% higher than that seen after inhalation via the p-MDI. However, with Turbuhaler®, a significantly larger fraction, 2.2 times, of the metered-dose was deposited in the lungs than with p-MDI (Thorsson et al, 1994).

2.8.2.1.3 30 minutes drug excretion post-inhalation

30 minute urinary drug excretion post-inhalation is a simple, non-invasive technique that has been developed for the evaluation of the relative bioavailability of salbutamol to the lung. The method is based on renal clearance of salbutamol and its metabolites (Hindle and Chrystyn, 1992). In crossover studies comparing different inhalers or inhalation methods the urinary recovery of unmetabolised salbutamol in the first 30 minutes post treatment is indicative of the relative bioavailability of salbutamol in the lung. The cumulative 24 hour urinary recovery of salbutamol and its sulphate conjugate metabolite allows an estimate of the relative systemic availability of the inhaled dose (Hindle and Chrystyn, 1992).

A more recent study by Hindle et al (1997) compared the relative bioavailability of salbutamol to the lung following inhalation via DPI and p-MDI devices. The study involved 10 healthy subjects that inhaled 2x 100 µg of salbutamol with 30 seconds separating each dose. Urine was then collected before drug administration, 30 minutes, and 24 hours after administration. The amount of salbutamol recovered at 30 minutes post-treatment was significantly ($P<0.001$) higher after inhalation from a DPI compared

to a p-MDI. The average amount of salbutamol renally excreted in the first 30 minutes following inhalation was 8.4 μg for DPI and 5.0 μg for p-MDI. The average amount of unmetabolised plus metabolised salbutamol recovered up to 24 hours post-treatment was 187.9 μg following inhalation with DPI and 137.6 μg following inhalation with p-MDI. The higher proportion of sabutamol recovered post-treatment with DPI compared with p-MDI indicates that the DPI may be a better device to deliver salbutamol to the lung (Hindle et al, 1997).

2.8.2.1.4 Gamma-Scintigraphy methods

Gamma scintigraphy is based on reformulating an existing inhaled product to incorporate a radiolabel. The radiolabelled formulation is then inhaled and the amount of radioactivity is determined following imaging by a gamma camera. There are two types of gamma scintigraphy, two-dimensional and three-dimensional imaging methods (Newman and Wilding 1999, Chrystyn, 2001). The two-dimensional method produces planer images of the radionucleotide, usually 99m technetium that is adhered to the drug formulation and after inhalation produces images identifying the drug deposition in the lungs (Newman and Wilding, 1999). The quantitative measurement of regional aerosol deposition in human lungs using two-dimensional gamma scintigraphy has proven to be useful in therapeutic and diagnostic aerosol studies. However, the disadvantage lies in the penetration index which is defined as the ratio of activity in a peripheral lung zone to a central lung zone. In two dimensional imaging the ability to discriminate between aerosol deposition in the large airways and alveolar region is reduced by the fact that the alveolar region overlies in the central zone of the large airways (Phipps et al, 1989).

Three-dimensional imaging method was recently introduced to address the disadvantages of the planer images. Such method is similar to the two-dimensional approach except that the gamma camera rotates through 360˚.This increases data

collection time, however, doses required are also much larger (40 times) than that required for the planar imaging and thus introducing formulation and preparation problems (Newman and Wilding 1999).

Nonetheless, positron emission tomography (PET) or three dimensional functional imaging techniques that provides accurate and highly specific information on dose, distribution, and kinetics of an inhaled or injected radiotracer in the lung (Dolovich and Labiris, 2004). Three-dimensional imaging also offers several advantages over the two dimensional imaging. First, it provides increased image resolution allowing greater detail and accuracy of the regional distribution of the drug. Additionally, an in vivo estimate of large and small airway deposition of the inhaled drug can be made using direct-radiolabeled drug molecules (Dolovich and Labiris, 2004).

Gamma scintigraphy method was used to assess the pulmonary deposition of the budesonide from a Monodose inhaler (Miat, Milan, Italy) and Turbuhaler® in twelve asthmatic patients. Patients inhaled from each device with maximal or sub-maximal inspiratory effort of 90 or 45 L/min from the Monodose inhaler inhaler and 60 or 30 L/min from Turbuhaler®. The formulations were radiolabelled with ^{99m}Tc, and deposition of budesonide was quantified by gamma scintigraphy. Mean average of whole lung deposition for the Monodose inhaler was found to be independent of inspiratory effort, while lung deposition for the Turbuhaler® fell significantly with decreasing inspiratory effort. The plasma concentrations of budesonide showed the same trends as the whole lung deposition data (Ball et al, 2002).

The long term safety and the expensive study cost are considered disadvantages of gamma scintigraphy (Chrystyn, 2001).

2.9 Patterns of Particle size Distribution

Studying particle size of an inhalation aerosol is important, because only those particles that fine enough (below approximately 5μm) can gain access to the airways and be available for therapeutic effect in the lungs (Berlinski, 2006). In addition, respiratory disease also contributes to the complexity of drug delivery to the lungs due to changes to the architecture of the lung through alterations in burification angles and obstruction of the airways due to mucus accumulation. These changes modify the deposition and distribution patterns of aerosols. As a result, airway obstruction diverts inspired air to unobstructed airways, thus only a limited dose of the drug is deposited in the obstructed areas that need to be reached in order to achieve the optimal therapeutic effect of the drug.

In line with this, there are important parameters that must be achieve in order to improve inhalation product performance. These include the amount of medication delivered to the patient and the effective aerodynamic particle size of the medication. As illustrated in Figure 2.13, to estimate the amount of drug actually available for deposition in to the lungs, a combination of total dose emitted and particle size as referred as fine particle mass (<5μm) are important since it is generally accepted that particles with aerodynamic diameter between 1 and 5μm have the highest probability of depositing in the lung (Berlinski, 2006). Johnson et al (1989) demonstrated that the bronchodilation response of cumulative dose of ipratropium bromide delivered either as a 3.3 μm or 7.7 μm aerosols was identical, whereas the response to salbutamol was significantly greater with the finer (3.3μm) aerosol, suggesting targeting drug aerosol to the location of those specific receptors in the lung does influence its effectiveness. Zanen et al (1996) also examined the optimal particle size of anti-cholinergic drug ipratropium bromide and β2 agonist salbutamol on equal doses of three different sizes of monodisperse aerosols of 1.5 μm, 2.8 μm, 5 μm. The finding suggested that small

particles penetrate more deeply into the lungs hence producing more effective dilation of small airways whereas larger particles were retained in the upper airways. The 1.5 μm aerosol induced significantly less bronchodilation than 2.8 μm aerosols, suggesting that these fine particles may be deposited too peripherally and not targeting the smooth muscle in the alveolar region. More recently it has been suggested especially for patients with obstructive lung disease that all particles should ideally be within the 2-3 μm range (Terzano, 2001). Furthermore, in order to optimize inhalation product performance, efforts have been focused on maximizing the amount of drug in this size range. However, it is apparent that many other factors can contribute towards the variation in the generation, characterisation, and utilisation of fine particle mass (Figure 2.13).

In MDI efficient inhalation requires successful coordination of actuation with inhalation. In practice, this translates into a slow and deep inhalation upon actuation that lasts approximately 2 seconds in a child and 5 seconds in an adult. However, failure to successfully achieve this as consequences of only small fraction of the drug dose being deposited in the lung. Typically, only 10-20% of the emitted dose is deposited in the lung (Newman and Clarke, 1992). The high velocity and the large particle size of the aerosol spray causes 50-80% of the drug dose to impact in the oropharyngeal region (Crompton, 1982), whereas smaller particles (1.5 μm) show little difference in the lung and oropharyngeal deposition during either fast or slow inhalation (Usmani et al, 2005). Hand-mouth coordination is another major obstacle in the optimal use of MDI products. Studies by Crompton et al (1982) have reported that 51% of patients experience problems coordinating actuation of the device with inhalation, 24% of patients halted inspiration upon actuation of the aerosol into the mouth, and 12% of patients inspired through the nose instead of mouth when the was actuated into the mouth. The delivery efficiency of a MDI studied by Bennett (1995) et al and Dolovich et al (1983)

demonstrated that for any particle size between 1 and 5μm, fast inhalation resulted in decreased total lung dose deposition and penetration into peripheral airways. However, when aerosols are inhaled slowly through a MDI, deposition by gravitational sedimentation in the peripheral regions of the lung is enhanced (Newman et al, 1982). Increasing tidal volume and decreasing respiratory frequency have also been shown to increase peripheral deposition. As the inhaled volume is increased, aerosols are able to penetrate more peripherally into the lungs (Pavia et al, 1979). This study shows the effect of the mode of inhalation of aerosols on the depth of deposition in the lungs of 50 patients with airways obstruction using a method of radioactive tracer particles. The finding revealed that the penetration of particles is directly associated with volume inspired per breath and forced expiratory volume in one second suggesting that to enhance depth of deposition of therapeutic aerosols in the lungs full breaths of the aerosol should be inhaled slowly followed by a breath-holding pause which 'freezes' some particles at the furthest point of penetration (Pavia et al, 1979). Moreover, a period of breath holding on completion of inhalation enables particles which penetrate the periphery to be deposited in that region, instead of being exhaled during the expiratory phase (Newman et al, 1982). DPI was designed to eliminate the co-ordination difficulties associated with the MDI. Before inhalation, the formulation of DPI has no potential lung deposition. It is the patient's inhalation that actuates the powder in a DPI into an emitted dose of particles with the appropriate characteristics for deposition in the lungs. Thus, dispersion of the drug powder into respirable particles depends on the creation of turbulent air flow in the powder container, which causes the aggregates to break up into the respirable particles (Concessio et al, 1991). Moreover, each DPI has a different air flow resistance that estimates the required inspiration effort (Richards and Saunders, 1993, Chrystyn, 2003). The higher the resistance of the device the more difficult it is to generate an affective inspiratory flow to achieve the maximum

dose from the inhaler. However, deposition in the lungs tends to increase when using high resistance inhalers (Clark and Hollingworth, 1993). This was established in studies using DPI with high resistance such as Easyhaler® where lower inspiratory flow were required to achieve the maximum dose from the inhaler while for inhaler devices with lower resistance such as Accuhaler® and Novohaler® required faster inhalation flow (Chrsytyn and Price, 2009). In line with this, lung deposition varies amongst the different DPI. Approximately 12-40% of the emitted dose is estimated to be delivered to the lungs with 20-25% being retained in the device. When a patient inhale through a DPI failure to use a fast inhalation from the start results in emission of particles that are too big to be deposited in the lung and so the dose is deposited in the oropharyngeal region and subsequently swallowed (Broeders et al, 2001). If the inhalation is too fast, the powder may not disintegrate before leaving the inhaler, which also leads to greater oropharyngeal deposition. With most DPI, drug delivery to the lungs is enhanced by fast inhalation. This was demonstrated in studies by Borgstrom et al (1994) who showed that increase in inhalation flow rate from 35 to 60 L/min increased the total lung deposition of terbutaline from 14.8% of the nominal dose to 27.7%. This is in contrast to the MDI, which requires slow inhalation and breath holding to enhance lung deposition of the drug.

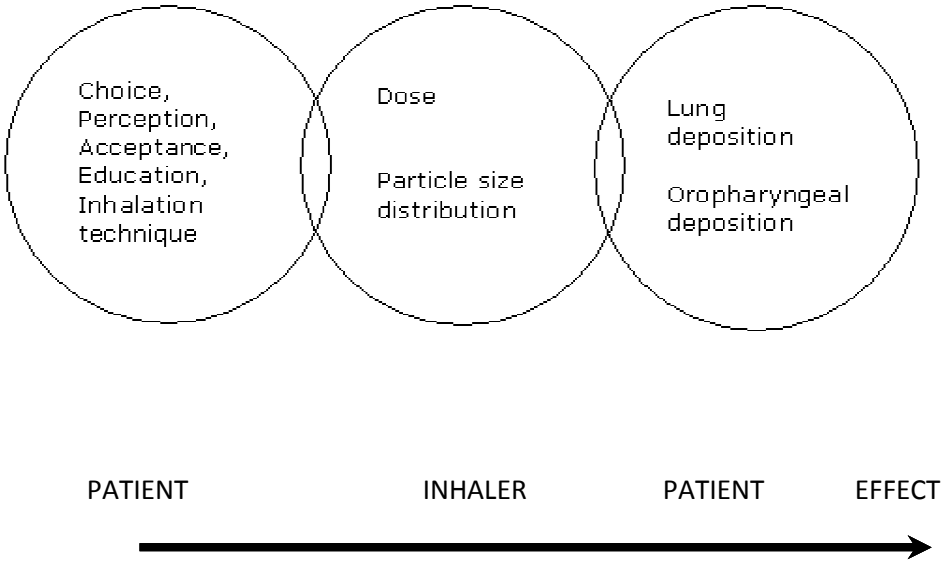

Figure 2.13: The relationship between the patient and the inhaled product (Adapted from Chrystyn, 2009).

2.10 Flow dependent dose emission

2.10.1 In vivo studies

Another important factor influencing inhalation product performance is the inspiratory flow rate generated by the patient. Because inspiratoty flow rate may vary from dose to dose in a given patient and also between patients (Borgstorm and Newman, 1993, Engel et al, 1992, Pedersen et al, 1990) its effect on inhalation device performance and moreover the potential to influence clinical outcome may also be altered.

In the past many clinical studies comparing the efficacy of MDI and DPI products have been conducted in which monitoring the inspiratory flow rate of patients was minimal (Hetzel and Clark, 1977, Zainudin et al, 1990) hence making it unclear if variabilities reported in the studies were influenced by the lack of control of the inspiratory flow rate.

More recently, studies have been carried out in which inspiratory flow rate was controlled and drug deposition and clinical outcome were compared between MDI and DPI.

MDI devices are stable, this is primarily attributed to its design since the doses determined by a metering valve. Furthermore, MDI devices have very fast delivery systems resulting in an external flow rate ranging from 30 to 80 L/min that is applied to the aerosol product. As a result MDIs are expected to have little effect on the total drug dose emitted that reaches the lungs (Smith et al, 1998).

Nonetheless, studies have revealed the need for slow and deep inhalation flow rates whilst inhaling from a MDI. Studies by Newman et al, (1991) have shown that low inhalation rates of 30 L/min were more beneficial to patients. In separate studies by Bennett et at, (1987) and Dolovich et al (1981) it was also demonstrated that increase in the inhalation flow rate resulted in decrease in the in the lung dose deposition and penetration into peripheral airways of the lung. Fast inhalation of 60 L/min resulted in reduced peripheral deposition because aerosols were more readily deposited in the oropharyngeal region.

These results are in agreement with Scheuch and Siekmeier (2007) who demonstrated that the alveolar deposition of aerosol particles continuously decreases with increasing respiratory flow rate (Figure 2.14).

A study of 30 random asthmatic patients revealed that MDI were used at peak inspiratory flow rates ranging from 50 to 400 L/min. This study design was chosen since most asthmatic patients tend to inhale too rapidly. A period of breath-holding was used, which resulted in an increase in the numbers of particles deposited in the lungs. A 4 seconds breath-hold after inhalation of 500 μg Terbutaline at a slow inspiratory rate of 25 L/min resulted in significantly less bronchodilation than after 10 seconds of breath-

holding. No additional bronchodilation was produced by extension to 20 seconds (Goodman et al, 1994).

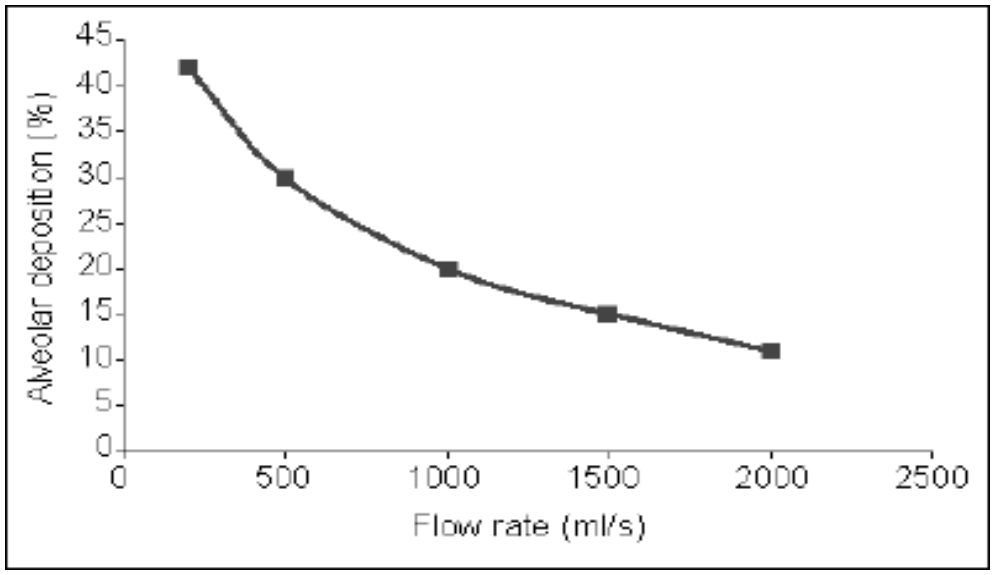

Figure 2.14: demonstrate the effect of flow rate and inhaled volume on the alveolar deposition of aerosol particles (Scheuch and Siekmeier, 2007).

DPI devices have also been extensively analysed in vivo. In such studies results demonstrated the importance of inspiratory flow rate on the performance characteristics of DPI in the clinical outcome (Pedersen et al, 1990, Engel, 1989, Engel et al, 1992, Dolovich et al, 1988Munzel et al, 2005).

One of such studies carried out by Newman et al (1991) reported that when 10 asthmatic patients inhaled terbutaline from a Turbuhaler® at slow (28.3 L/min) and fast (60 L/min) rates, significantly more drug was deposited in the lung using a fast inhalation flow rate than with the slow rate. The data showed that the inhalation of terbutaline via the Turbuhaler® at the fast flow rate resulted in a significant ($p<0.01$) increase in the lung deposition, which was matched by a significant decrease in deposition in the oropharnyx ($p<0.01$), and percentage of the dose left in the mouth piece and in the exhaled air were similar for the two inhalations. The dependency of emitted dose from a DPI device on the inspiratory flow rate has

been shown to produce clinically differences across a range of different devices (Auty et al, 1987, Pedersen et al, 1990).

In line with this, separate studies using Gamma scintigraphy to measure the in vivo drug deposition in healthy subjects at different flow rates of 45, 60, and 90 L/min for the budesonide Novohaler® device and 60 L/min Turbuhaler® device showed variability of the lung deposition, which was clearly lower with the Novohaler® than with the Turbuhaler®. The difference was statistically significant (p = 0.029) at a comparable targeted flow rate of 60L/min (Munzel et al, 2005). The differences in DPI devices are further compounded by the inability of some patients in achieving a fast rate during routine use of DPI (Chrystyn, 2003, Pedersen et al, 1986, Pedersen and Steffensen, 1986). This was revealed in studies carried out by young children and those with severe obstructive disease, which showed that they are most likely to have problems using a fast inhalation flow. This is indeed problematic since DPIs are highly dependent on achieving a certain inspiratory flow, and then there is a risk of reduced bronchodilation response during episodes of acute wheezing or in patients with low lung function (Pendersen, 1987). For instance in a situation where the patient is unable to generate sufficient flow, the DPI devices will perform differently. When using a common inhalation flow rate of 105 L/min, a patient with obstructive disease can still generate his particular flow rate whilst using an Aeroliser®, but only 63 L/min through a high resistance Turbuhaler®. In studies that followed, attempts were made to demonstrate the effects of one and two inhalations on dose emission at varying flow rates. Studies by Abdelrahim et al (2009) were design using Turbuhaler® as this has a very high resistance device hence patient inhalation flow when inhaling would be low. The total emitted dose of 500 μg terbutaline sulphate from a Turbuhaler® was determined using inhalation flow rates of 10 to 60 L/min with inhalation volume of 2 and 4 L and using a DPI sampling apparatus after one and two inhalations. The relative lung and systemic

bioavailability of terbutaline from Turbuhaler® when used by healthy subjects and COPD patients were determined after one and two inhalations at slow and fast inhalation flows using a urinary terbutaline pharmacokinetic method. Results showed that the nominal dose from the one and two inhalations increased significantly ($p < 0.05$) with the increase of the inhalation flow at both 2 and 4 L inhalation volumes. Additionally, the relative lung and systemic bioavailability after one inhalation at fast inhalation flow were significantly higher ($p < 0.01$) than at slow inhalation flow in both healthy subjects and patients. Also the healthy subjects results were significantly higher ($p < 0.05$) than the COPD patients after one inhalation. However, after two inhalations there was no significant difference between slow and fast inhalation flow or healthy subjects and COPD patients. This study indicates the relevance of inhaling twice and as deep and hard as possible from each dose from Turbuhaler. Especially, for patients with low inspiratory flow and limited inhalation volume as they may not receive much benefit from one inhalation (Abdelrahim, 2009).

2.10.2 In vitro studies

The influence of inspiratory flow rate on the fine particle mass and the particle size distribution for MDI and DPI products has also been extensively studied in vitro.

In such studies MDI devices showed more reproducible dosing characteristics independent of inhalation flow rate (Smith and Schultz, 1996). This stability in MDI device is primarily attributed to its design since the doses are determined by the metering valve. MDI devices also benefit from a very fast delivery system with external flow rates ranging from 30 to 80 L/min that is applied to the aerosol product. As a result MDI are expected to have little effect on the total drug dose emitted that reaches the lungs (Smith et al, 1998).

Comparative studies by Ross and Schultz (1996) the effect of inhalation flow rates (30 and 60 L/min) on the particle size distributions of inhalation for albuterol, beclomethasone, budesonide, and terbutaline in both MDI and DPI were analysed. Results showed that although the medication delivery, MMAD, and fine particle dose were independent of drug or device used, the MDI products had a more reproducible respirable dose than the breath-actuated DPI products tested as a function of inhalation flow rate.

The performance of MDI and DPI inhalers containing the same drug and strength were also analysed at different simulated inspiratory flow rates (30 L/min, 60 L/min and 90 L/min). Results demonstrated that DPI was significantly more dependent on impaction flow rate than MDI devices (Feddah et al, 2000).

In separate studies by Karen et al (1998) no differences were observed in total drug mass delivered by MDI when the inhalation flow rate was increased from 30 to 55 and 80 L/min. Conversely, a significant increase was observed in the drug mass delivery through a DPI when the flow rates were increased from 30 to 55 L/min, the amounts of the drug deposited in the Anderson cascade with an increase in flow rates from 30 to 55 L/min from DPI was 59% and 94%, respectively.

However, because of the high velocity of inhaled particles through MDI this leads to high oropharyngeal deposition, hence the need for slow and deep inhalation flow rates whilst inhaling from a MDI (Feddah, 2001).

In a computational study, efforts were made to study the effects of flow rate on aerosol penetration efficiency for MDI applications. Measurements show that airflow rate has an apparent and significant effect on particles deposition in the oral airway. The reduction of the airflow rate from 90 L/min to 30 L/min tripled the penetration efficiency of the MDI (Fadl et al, 2007).

With regards to DPI, several in vitro studies have been reported on inhalation flow dependency of DPIs (Dolovich et al, 1988, Borgstrom et al, 1994). The design of different DPI devices is optimised to emit their formulation as a respirable dose during inhalation. Because differences in formulations the resistance in each type of DPI is also different. Therefore, this makes the inhalation flow rate through a DPI very important in generating the respirable dose since all patients will inhale at a different rate (Chrystyn, 2003).

In vivo studies with gamma scintigraphy studies demonstrated greater total lung deposition following inhalation of budesonide from a Turbuhaler using rates of 36 and 58 L/min. The mean (SD) total lung deposition was 14.8 (3.3) and 27.7 (9.5) %, respectively (Borgstrom et al, 1994). In separate gamma scintigraphy studies using terbutaline at inhalation flow rates of 28 and 57 L/min through a Turbuhaler, results demonstrated that the total lung deposition was 9.1 (1.5) and 16.8 (2.6) %, respectively (Dolovich et al, 1988).

When a patient inhales through a DPI, the turbulent energy inside the device is created by the pressure drop that results from the interaction between the patient's inspiratory flow and the resistance of the DPI device. For each DPI device there is a minimum threshold energy required at which the disintegration is sufficient to provide a dose with the potential to produce an aerosol cloud, which contains a high fraction of drug particles with the desired particle size (<5μm) for lung deposition (Haughney et al, 2010). There are various different disintegration principles which are influenced by the drug formulation and the design of DPI devices. They may vary from simple (Rotahaler®, Diskhaler®) to twisted powder channels (Turbuhaler®). The applied disintegration concept in the design of the DPI largely determines the resistance to airflow of the inhaler device.

Inhalers without a recognisable disintegration principle (Figure 2.15), such as Diskhaler® and Accuhaler®, often have low resistance airflow. As a consequence of non-specific disintegration system, the fine particle fraction generated by the inhaler is low. Due to the low resistance to airflow, larger variations in the peak inspiratory flow rate are expected. Conversely, the fine particle dose emitted is relatively constant through a range of different inspiratory flows at a low level, with only a slight tendency to increase as the inhalation flow rate increases (De Boer et al, 1996).

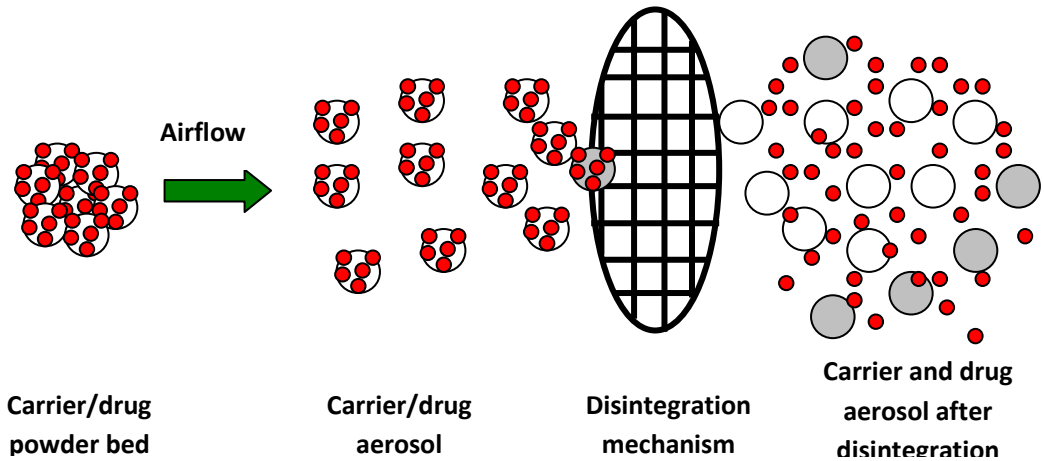

Figure 2.15: Schematic diagram of the disintegration of micronised drug particles from carrier crystals through a non-specific disintegration system.

In devices with specific disintegration systems (Figure 2.16) such as Turbuhaler® the pressure drop occurring within the device during inhalation elevates extensively with increasing airflow rates.

Thus the more resistance there is inside the inhaler then the lower will be the inhalation flow for a set inspiratory effort. The high resistance to airflow rates limits the range of

possible inhalation flows. However, due to higher disintegration efficiency, the fine particle dose is higher compared to the non-specific disintegration (Srichana et al, 1998, Hawksworth et al, 2000, Koning et al, 2002).

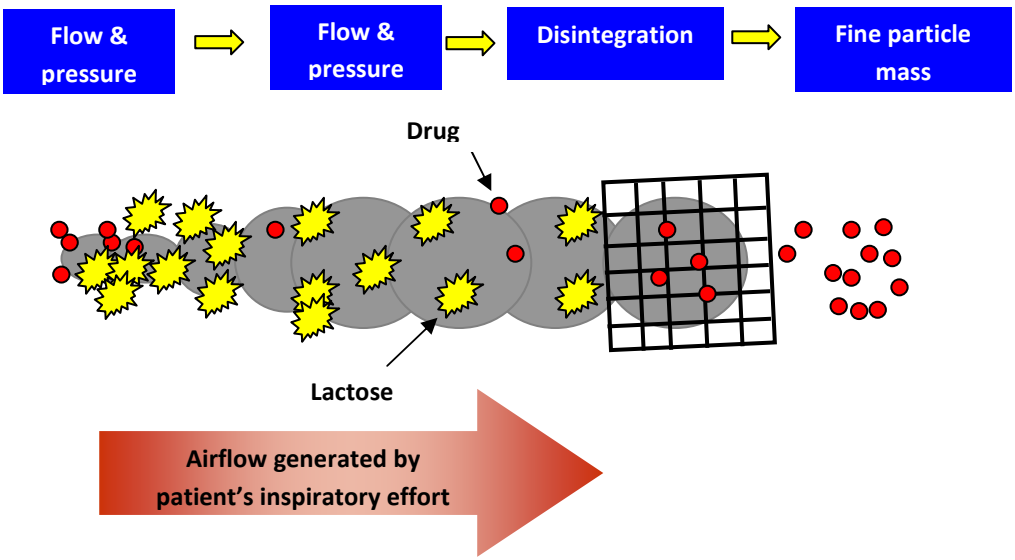

Figure 2.16: Schematic diagram of the disintegration of spherical pellets through a specific disintegration mechanisms.

Therefore, due to the intrinsic resistance of DPI devices, the patient has to generate a particular inspiratory flow, which is determined by the type of DPI used. About the same amount of inspiratory flow rate produces a considerably lower flow through a device with high intrinsic resistance compared to one with low resistance.

Studies by Chew and Chan (2001) focused on two DPI devices with different intrinsic resistance. Foradil Aeroliser® and Oxis Turbuhaler® are two DPI devices available for formoterol. The resistance for the air flowing through an Aeroliser® is significantly lower than that of the Turbuhaler® (Clark and Hollingworth, 1993).

When the performance of the two DPI devices were compared in producing formoterol aerosol, results showed that Aeroliser® has a slight dependence of the emitted dose on the air flow, with average dose increased from approximately 80% (at 30 L/min) to 90% (at higher flows), with Turbuhaler® delivering only 60% (Chew and Chan, 2001). Additional studies by Weuthen et al, 2002) comparing the performance of DPI devices also demonstrated that Turbuhaler delivers about 16% less formoterol aerosol than Aeroliser®. Aeroliser® delivered more constant doses of approximately 80% of the nominal dose at flow rates of 60% or higher. Such measurement of specific air flow resistance for Aeroliser® suggests that they are favourable for children or those suffering from lung disease. Conversely, the higher resistance of the Turbuhaler®, influences the ability of inhaling through it, thus making it more problematic, especially for asthma suffers (Weuthen et al, 2002).

Additionally, studies were also carried out looking at a range of different DPI devices as result of intrinsic resistances. The design differences were once again shown to affect parameters such as the internal resistance of a device which in turn affects the flow rate achieved through the DPI. Turbuhaler® and Diskus® were compared for the delivery of budesonide and fluticasone propionate respectively. The Diskus® delivered 87–93% of the label claimed dose while the Turbuhaler® delivered 40–58% (Hill and Slater, 1998).

Additionally, flow rate has been shown to directly affect the proportion of the nominal dose and also the fine particle dose of the aerosol released from different types of DPI. This effect is more significant with some DPIs than others. For instance, at a constant flow rate 60 L/min, the fine particle dose varied by 40% of the nominal dose in the Turbuhaler® and < 10% with the Spinhaler® (de Boer, 1996). Furthermore, it was observed that even within the type of DPI device, flow rate directly affects both proportion of nominal dose and fine particle dose. In line with this, increasing the flow rate of air through a budesonide/formoterol Turbuhaler® from 30 to 60 L/min and 90

L/min resulted in increased budesonide nominal dose from 37.5% to 64.4% and 107.4% respectively (Tarsin et al, 2004) and consequent increase in formoterol nominal dose. Increases in nominal dose were parallel with increase in flow rate. Similarly, the fine particle dose of both drugs also increased sharply when the flow rate was increased from 30 to 60 L/min. Moreover, results also show that intra-inhaler dose emission can also be erratic as observed with Tubuhaler® but not with the Diskus®. Studies have shown that Diskus® has a higher degree of dose consistency through the life of the device than that achieved in multi-dose reservoir devices (Figure 2.17).

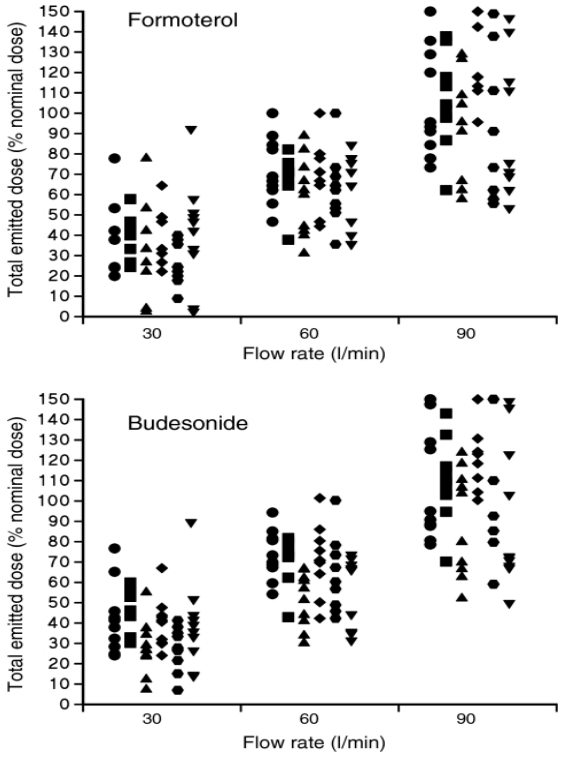

Figure 2.17: The amounts of budesonide and formoterol (expressed as a percentage of the labelled emitted dose) emitted from each dose of the six inhalers tested using in vitro inhalation flow rates of 30, 60 and 90 L/min (Adapted from Chrystyn etal , 2007).

Moreover, the particle size generated by a particular device may be also influenced by the inspiratory flow. In vitro studies by Weuthen et al (2002) were carried out to test the efficiency of two different DPI systems for the application of formoterol in the lungs. Particle size distributions for each device were measured at four different flow rates (28.3, 40, 60, and 80 L/min) using an Andersen Cascade Impactor and MMAD of the dispersed powder and deposition of the drug in the respiratory tract was determined. The results showed that Turbuhaler® delivers smaller particles as the Aerolizer®, thus suggesting that formoterol reaches deeper into the lungs. However, at inspiratory flow rates of 30 L/min the particle size generated through the Turbuhaler® were twice as large as particles from a flow of 40 L/min (Weuthen et al, 2002).

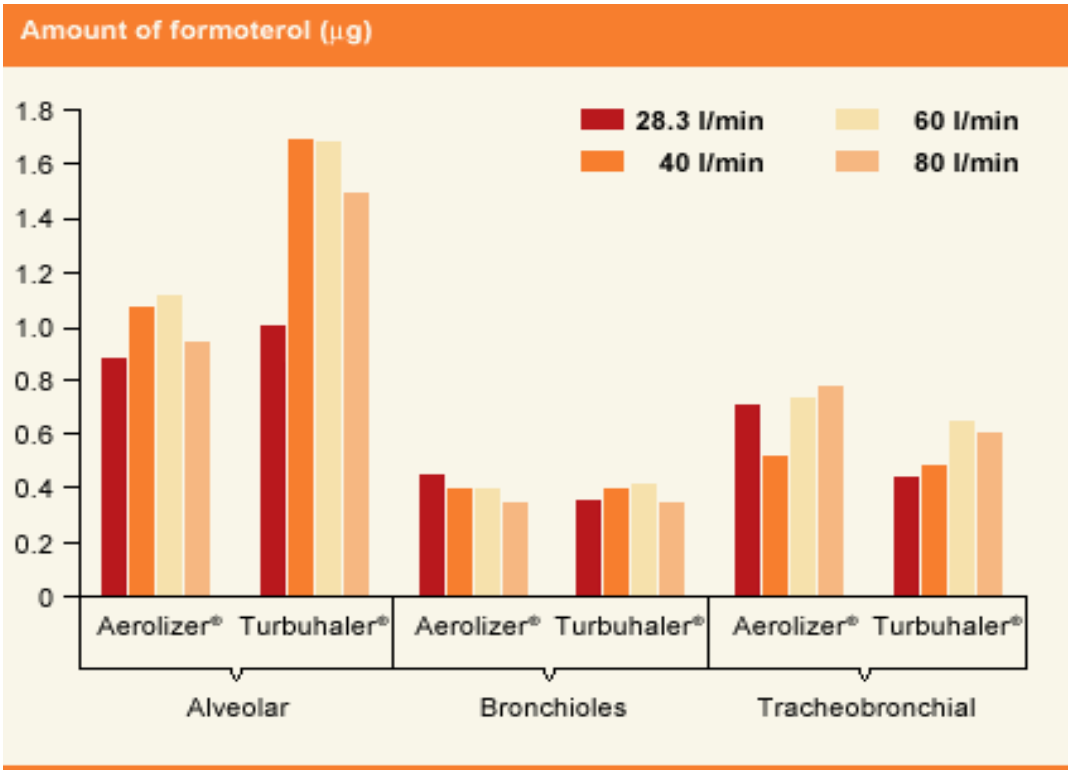

Figure 2.18: In vitro testing of two formoterol DPI (Turbuhaler and Aeroliser) at different flow rates (Weuthen et al, 2002).

In separate studies assessing the performance of Aeroliser® and Turbuhaler® in producing fine drug particle at varying inhalation flow rates, results demonstrated that at flow rates of 30 and 60 L/min both inhalers generate significantly less fine particles in the aerosols ranging from 0.5 to 1.7 μm), with Turbuhaler® producing significantly lesser amounts that the Aeroliser® at 30 L/min. At higher flow rates of 90 and 120 L/min both inhalers generated very similar amounts of fine particles of approximately 4, 3, and 1.7 μm in the aerosol discharged (Figure 2.18) (Weuthen et al, 2002).

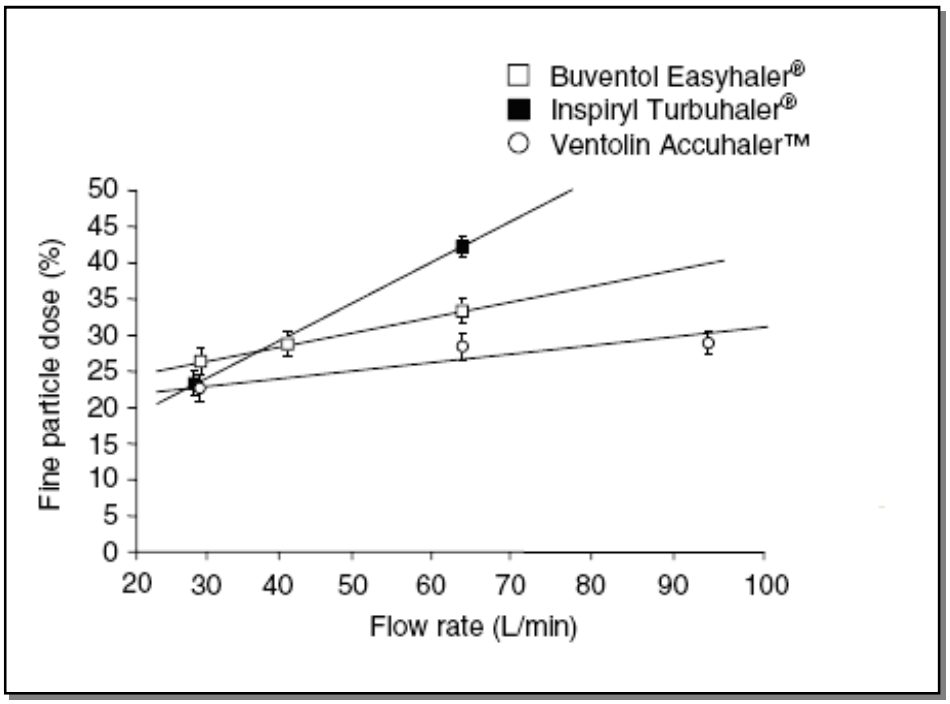

Figure 2.19: The influence of inhalation flow on the fine particle dose (expressed as percentage of label claim [%LC]) emitted from an Accuhaler™, an Easyhaler® and a Turbuhaler® (Adapted from Palander et al., 2000).

In studies by Palander et al (2000) attempts were made to demonstrate the properties of flow dependent dose emission from three different DPI devices (Figure 2.19). While

some DPI devices such as the Turbuhaler® have been shown to produce large differences in the emitted dose and fine particle fraction (FPF) as a function of change in the flow rate (Tarsin et al., 2004, 2006, Palander et al, 2000), the Easyhaler® and the Accuhaler® devices are shown in Figure 2.19 to produce less variation in the emitted dose and fine particle fraction upon increasing the flow rate between 30 and 90 L/min (Palander et al, 2000).

Tarsin et al (2004) also measured the in-vitro dosage of the fine particle dose from 100/6 and 200/6 Symbicort Turbuhaler® for budesonide and formoterol, respectively at different flows of 30, 60, 90 L/min. The data shows that the amounts of budesonide and formoterol emitted from the Symbicort® inhalers were affected by the increase in inhalation flow. Additionally, the results demonstrate that the average total dose emissions at 90 L/min were greater than 100%. Each determination at 90 L/min was carried out after doses had been discharged using flow rate of 60 L/min. The data obtained suggest that at 60 L/min the total dose emission is not 100%. Thus the total dose emission of >100% at 90 L/min suggests that when using high flows some residual dose from the previous inhalation may also be inhaled. The study also showed that from the Turbuhaler® there was an intra-inhaler variability of the dose emitted from the same inhaler and that there was also intra-device variability.

In separate studies the Clickhaler®, a more recent DPI device to reach the market, results showed little variation in the clinically relevant range of flow rates from 15 to 60L/min (Newhouse et al, 1999).

There are also reports that the mass median aerodynamic diameter (MMAD) decreases as the inhalation flow increases (Tarsin et al, 2003). Theoretically, if the fine particle dose is not significantly affected by flow then central lung deposition could be increased with higher inspiration rates. However, it has been shown that, even though the dose

emission of the Accuhaler® was not significantly affected by increasing flow rates, there was a decrease in the overall MMAD. The change in the MMAD with inhalation flow rate was similar for both Accuhaler® and Turbuhaler® devices (Tarsin et al, 2006). Similarly, in separate studies examining the influence of inspiratory flow rate on the fine particle mass and the MMAD for the DPI products revealed that by increasing the inspiratory flow rate from 30 L/min to 60 L/min and 90 L/min, the fine particle mass significantly increased by 17% and 75% for Acchuhaler® and by 1.2 and 2.2 fold for Turbuhaler® and by 43% and 129% for Rotahaler®, respectively. Furthermore, a significant decrease in the MMAD was observed on the aerosolised particles calculated from the particles entering the impactor by increasing the flow rate from 60 L/min to 90 L/min for all the DPI products tested (Table 2.3). This decrease in MMAD would counteract the greater potential for more central deposition of particles when using a faster inhalation flow rate (Feddah et al, 2000).

Table 2.3: Fine Particle Fraction (FPF) from the labeled dose and the MMAD of Flixotide Accuhaler® (FLA), Pulmicort Turbuhaler® (PLT) and Becotide Rotahaler® (BCR) at different flow rates (Adapted from Feddah et al, 2000)

Flow rate (L/min)	FLA		PLT		BCR	
	FPF±SD	MMAD±SD	FPF±SD	MMAD±SD	FPF±SD	MMAD±SD
30	19.16±2.6	3.2±0.1	12.43±2.8	3.2±0.3	10.6±1.2	4.1±0.76
60	22.44±1.4	3.1±0.4	28.43±2.3	2.9±0.2	15.2±1.0	3.7±0.6
90	36.87±3.4	1.95±0.2	40.74±5.5	1.8±0.15	24.3±2.3	2.3±0.23

Mean ± SD for five replicates

The fine particle mass is mass less than 4.0μm

A modification of the aerosol sampling methodology was used to obtain the nominal dose and fine particle dose measurements at variable flow rates. The technique consists of an Electronic Lung that employs a variable sampling flow rate which is derived from *in vivo* recordings of patient breathing patterns. Hence, the technique allows inhalation profiles from different patient groups to be recorded using a pressure-sensitive device and replayed on the in vitro method. This technique has been used in studies to simulate the breathing pattern of asthmatic children aged 4–8 years. Subsequently, the nominal dose of fluticasone propionate via the Diskus® was compared with that of budesonide delivered via the Turbuhaler® (Bisgard, 1998). Such results were in correlation with those obtained by pharmacopoeial methods and showed that 87–89% of the label claim was emitted from the Diskus® compared with 56–62% from the Turbuhaler®. Interestingly, the fine particle dose from the Diskus® was slightly lower than that from the Turbuhaler® (15–18% compared with 21–32% respectively). Overall, the results showed that the Diskus® delivered a more consistent dose across the varying inhalation patterns than the Turbuhaler® (Bisgaard, 1998). This technique has been used to estimate the dose that severe asthmatics would receive when inhaling from a Diskus® (containing 500 mcg fluticasone propionate with 50 mcg salmeterol) and a Turbuhaler® (containing 200 mcg budesonide and 6 mcg formoterol) (Tarsin, 2006). Results demonstrated that the range of inspiratory flow rates generated by the patients through the two devices were contrasting, with Diskus® showing less of an inhalation flow rate effect on the dose emitted while Turbuhaler® showed a significant effect on dose emitted. Additionally, it was also observed that there was an inverse relationship

between inspiratory flow rate and MMAD of aerosol released from both DPIs (Figure 2.20). The change in the MMAD with inhalation flow rate was similar for both devices (Tarsin, 2006).

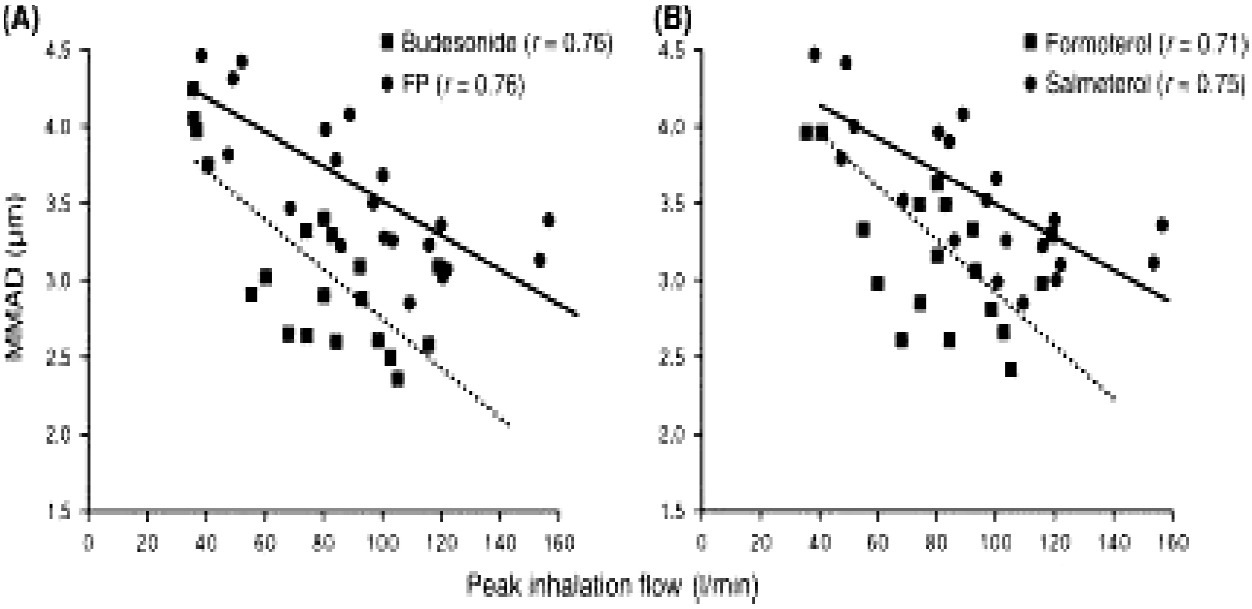

Figure 2.20: The MMAD with a peak inhalation flow (A) budesonide and fluticasone propionate and (B) formoterol and salmeterol (Chrystyn, 2007).

The aerodynamic properties of the dose emitted and the inhalation flow dependent characteristics from a DPI have also been determined using a novel methodology to measure these at <28.3 L/min. This incorporated the use of the original Andersen Cascade Impactor (ACI) and another adapted with a mixing inlet, which allows inhalation flows through the DPI from 5 to 60 L/min. The mean fine particle dose for formoterol from a Turbuhaler® using the mixing inlet method at 10, 20, 28.3, 40 and 60 L/min was 0.55, 1.39, 1.80, 2.88 and 5.86 μg and the MMAD was 6.6, 6.0, 5.4, 5.1 and 2.8 μm, respectively. Although the accuracy of the original ACI <28.3 L/min was unknown, the ACI with the mixing inlet allowed the determination of the in vitro dose

emission properties of the DPI at flows <28.3 L/min whilst maintaining a constant flow through the ACI, which is highly desirable focusing attention to the lowest inhalation flow required for a DPI (Nadarassan et al, 2010).

Table 2.4: Summary of studies investigating flow dependent dose emission.

MDI demonstrated more reproducible dosing characteristics independent of inhalation flow rate	Smith and Schultz, 1996
MMAD and fine particle dose were independent of drug or device but MDI dose more reproducible than DPI at different flow rates.	Ross and Schultz, 1996
DPI was significantly more dependent on impaction flow rate than MDI glucocorticoid products.	Feddah et al, 2000
No differences observed in total drug mass delivered by MDI at flow rates of 30, 55 and 80 L/min. A significant increase was observed in the drug mass with an increase in flow rates from 30 to 55 L/min from DPI was 59% and 94% respectively.	Karen et al, 1998
Slow inhalation through a MDI increase deposition by gravitational sedimentation in the peripheral regions of the lung.	Newman et al, 1982
Low inhalation rates of 30 L/min were more beneficial to patients.	Newman et al, 1991
Fast inhalation flow rate decrease drug dose deposition and penetration into peripheral airways of the lung.	Bennett et at, 1987; Dolovich et al 1981
The reduction of the airflow rate from 90 to 30 L/min tripled the	Fadl et al, 2007

penetration efficiency of the MDI.	
Alveolar deposition of aerosol particles continuously decreases with increasing respiratory flow rate.	Scheuch and Siekmeier, 2007
Several in vitro studies have demonstrated the inhalation flow rate dependency of DPI.	Schultz et al, 1992, Hindle et al, 1994, Hindle et al, 1995
In vivo studies on 10 asthmatic patients inhaling terbutaline from a Turbuhaler® showed significantly more drug deposition using a fast inhalation flow rate (60 L/min) than with the slow rate (28.3 L/min).	Newman et al 1991
DPI devices with different intrinsic resistance (Foradil Aeroliser® and Oxis Turbuhaler®) were compared. Aeroliser® show more dependency of the emitted dose on the flow rate, with 80% (at 30 L/min) to 90% (at higher flows), with Turbuhaler® delivering only 60%.	Chew and Chan, 2001, Weuthen et al, 2002
Turbuhaler and Diskus were compared for the delivery of budesonide and fluticasone propionate respectively. The Diskus delivered 87–93% of the label claimed dose while the Turbuhaler delivered 40–58%.	Hill and Slater, 1998
Turbuhaler®, Easyhaler® and Diskus® were compared for Intra-inhaler dose emission. Tubuhaler® showed some erratic properties but not with Easyhaleror the Diskus. Diskus had a higher degree of dose consistency.	Tarsin et al, 2004

In vivo drug deposition in healthy subjects at different flow rates of 45, 60, and 90 L/min for Novohaler® and Turbuhaler® were compared. The variability of the lung deposition was clearly lower with the Novohaler® than with the Turbuhaler®.	Munzel et al, 2005
The effect of increasing flow rates on the fine particle mass and the MMAD for the DPI products showed fine particle mass significantly increased by 17% and 75% for Acchuhaler® and by 1.2 and 2.2 fold for Turbuhaler® and by 43% and 129% for Rotahaler®, while a significant decrease in the MMAD was observed.	Feddah et al, 2000).
An in vivo technique to simulate the breathing pattern of asthmatic children aged 4–8 years was used and fluticasone propionate via the Diskus® was compared with that of budesonide delivered via the Turbuhaler®. 87–89% of the label claim was emitted from the Diskus® compared with 56–62% from the Turbuhaler®. The fine particle dose from the Diskus® was slightly lower than that from the Turbuhaler but Diskus® delivered a more consistent dose across the varying inhalation.	Bisgard, 1998
A novel technique using a cascade impactor with the mixing inlet to measure at >28.3 L/min was used to determine the in vitro dose emission properties of formoterol from Turbuhaler®. The mean fine particle dose for formoterol and the MMAD were similar to using both cascade impactor methods. This method focus attention to the lowest inhalation flow required for a DPI.	Nadarassan et al, 2010

First and second inhalation technique were investigated. The results indicate the relevance of inhaling twice from each dose from Turbuhaler®.	Abdelrahim, 2009

CHAPTER THREE

METHODOLOGY

3. MATERIAL AND METHODS

3.1 Instruments and Apparatus

3.1.1 High Performance Liquid Chromatography

High Performance Liquid Chromatography (HPLC) is a chromatographic technique that can separate a mixture of compounds, and is used in biochemistry and analytical chemistry to identify, quantify and purify the individual components of the mixture.

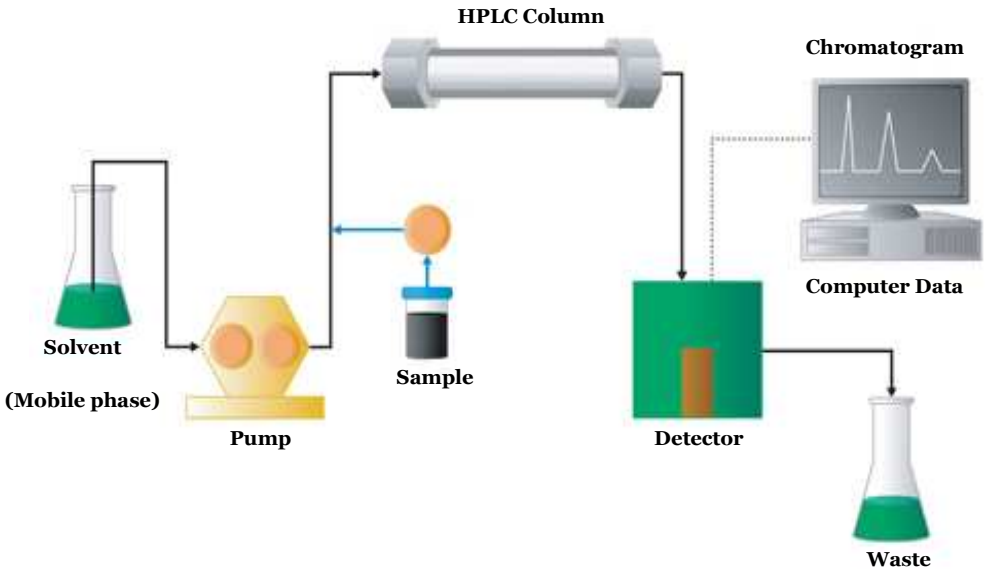

Figure 3.1: Diagrammatic illustration of High Performance Liquid Chromatography (Adapted from Waters, 2010).

HPLC utilises different types of stationary phase, a pump that moves the mobile phase and the sample to be analysed through the column, and a detector that provides a characteristic retention time for the sample. Sample retention time varies depending on the strength of its interactions with the stationary phase, the ratio and composition of solvents used, and the flow rate of the mobile phase. With HPLC, a pump provides the high pressure required to propel the mobile phase and sample through the densely packed column. The increased density arises from smaller particle sizes. This allows for a better separation on columns of shorter length when compared to ordinary column chromatography.

3.1.1.1 Pump and Autosampler

- Hewlett Parkard 1050 system with a multiple solvent delivery system containing an auto sample with a variable injection loop.

3.1.1.2 Detectors

- Variable wavelength UV/visible detector HP 1050 series.

3.1.1.3 Integrators

- Prime Multichannel Data Station (Version 4.2.0) (HPLC Technologies, Hertfordshire, UK).

3.1.1.4 HPLC columns and Guard columns

- Waters C18 (ODS2) Spherisorb® 250 mm X 4.6 mm i.d X 5 μm Waters (Milford, USA).
- Waters C8 Spherisorb® 250 mm X 4.6 mm i.d X 5 μm Waters (Milford, USA).
- Phenomenex C18 Guard Column, 10 mm X 4.6 mm i.d. X 5m (Phenomenex, Macclesfield, Chesire, UK).
- The analytical column was protected with a C18 (4 X 3 mm i.d) security cartidgre system (Phenomenex, Torrance, USA).

3.1.2 UV Spectrophotometers and Spectrofluorimetres

- Hewlett Packard (HP) 845A Diode Array Spectrophotometer connected to HP 89531A UV/VIS operating software. Solutions were placed in a 1cm pathlength quartz curve.

3.1.3 Apparatus used to determine dose emission

- GAST 1023 Pump, 0-100 L/min (GAST, Brook Hampton, Doncaster, UK)
- PR 4000 flowmeter (MKS Instruments, Andover, MA, USA)
- Dry powder controller Model TPK (Copley Scientific, Nottingham, UK; Instruments, Nottingham, UK)
- Dose Unit Apparatus for DPI (content uniformity) (Copley Scientific, Nottingham, UK)
- Glass fibre filter, 47 mm (Pall Gelman Sciences, Michigan, USA)

3.1.4 Apparatus and software used to evaluate the particle size

- Anderson Cascade Impactor (Copley Scientific, Nottingham, UK)
- Copley Inhaler Testing Data Analysis Software (CITDAS) version 2.00 (Copley Scientific, Nottingham, UK)

3.1.5 General laboratories apparatus

- Ultrasonic bath (Decon Laboratories, Hove, UK)
- Syringe filters, 0.2 μm pore size (Scientific Resources Inc., NJ, UK)used for filtering of samples prior to HPLC analysis
- Nylafo® nylon membrane filters, 47 mm, 0.45 0.2 μm pore size (Pall Gelman) for filtering HPLC mobile phases
- Filters for Anderson Cascade impactor (Copley Scientific, Nottingham, UK)
- Vortex mixture (Jencons Scientific, UK)
- Melter Toledo AL analytical balance (Leicester, UK)
- Thermo micro balance
- pH meter Accumet AB10 Basic (Fisher Scientific, Leicestershire, UK)
- Thermo micro balance

- Drier- nitrogen (Fisher Scientific, Leicestershire, UK)
- ELGA ultrapure water dispensing system (High Wycombe, UK)

3.2 Materials

3.2.1 HPLC mobile phase

3.2.1.1 HPLC grade solvents

- Acetonitrite (Fisher Scientific, Leicestershire, UK)
- Methanol (Fisher Scientific, Leicestershire, UK)

3.2.1.2 Buffer salts and additives

- Potassium dihydrogen phosphate (BDH Anal R grade R grade, Poole, Dorset, UK)
- Ortho Phosphoric acid (Fisher Scientific, Leicestershire, UK)
- Sodium dihydrogen phosphate (BDH)
- Disodium hydrogen phosphate (BDH)

3.2.2 Pharmaceutical material and inhalers

- Formoterol Fumarate Dihydrate (Sigma – Aldrich)
- Oxis Turbuhaler® (Astra Zeneca, Gloustechire, UK)
- Foradil Aerolizer (Schering) (Norvatis Pharma AG, Basel, Switzerland)
- p-MDI
- Easyhaler

3.3 Methods

3.3.1 Analysis of formoterol in aqueous samples

3.3.1.1 Preparation of buffers for mobile phase

Appropriate buffer salts were weighed out and transferred to volumetric flasks. To these ELGA water was added and the solutions were made up to the volume, before being mixed by inversion. The correct pH was achieved by adding a minimal volume of acid or alkali using a pasture pipette. The pH meter was calibrated at pH 4.0, 7.0 and 10.0 using commercially available buffer solutions.

3.3.1.2 Preparation of HPLC mobile phase

In order to avoid problems associated with the contraction that occurs upon the addition of organic solvents to aqueous solutions, these components were always measured separately and then mixed. The aqueous constituent was also adjusted to the correct pH before addition of the organic modifier. Following its preparation, the mobile phase was filtered under vacuum through a 0.45 μm nylon filter to remove any particulate that may block the HPLC system. The mobile phase was then de-gassed by sonication under vacuum for 10 minutes.

3.3.1.3 Preparation of aqueous standards

Appropriate amounts of formoterol were weighed and then dissolved in 70%v/v methanol/water to give a concentration of 10 mg/mL (Standard Stock A). Propranolol were also weighed and dissolved in 70%v/v methanol to yield a concentration of 100 mg/mL (Standard stock B).fifty mL of standard stock A and 5 mL standard stock B were mixed and diluted to 500 mL using 20 %v/v methanol (Standard stock C containing 1 mg/mL of formoterol and 10 mg/mL of propranolol).

Further dilutions were performed using appropriate dilutions with standard stock C.

3.3.1.4 Optimised HPLC conditions

High performance liquid chromatography (HPLC) provides one of the most efficient methods offering sensitivity and flexibility. However, it requires careful method development as any published HPLC method is specific to the condition used in its development.

In line with this, any major changes made, for instance the column, or manufacturer, or the brand of solvents used, can lead to dramatic alteration to the quality of the separation.

Therefore, this optimisation of the method is regularly required and involves the variation of the separation conditions in order to obtain the highest quality data.

The key factors governing the separation achieved are the organic modifier, column stationary phase chemistry, pH, buffer type and the inclusion of an ion pair regent (Wright et al, 1989).

In the present study the HPLC method optimisation was undertaken to achieve a system in which the formoterol peak was well resolved in the shortest time possible.

The selection of appropriate criteria depends on the ultimate goal of the separation and thus varies considerably between applications (Bourguignon and Massart, 1991). The degree of separation of the peaks is given as resolution. Therefore, no single criterion can be used to assess fully the adequacy of a separation, and a combination of effects is often used in the form of a resolution function value.

3.3.1.4.1 Selection of Suitable Stationary Phase

Initial trials were carried with various C18 columns to carry out the separation process due to formoterol being a weakly basic compound.

Furthermore, a similar stationary phase to that in studies by Assi et al (2005) for estimation of formoterol in in-vitro specimens were used. Waters C18 (ODS2)

Spherisorb was used in the present study due to more robust nature of its phase at low pH ranges (>2.5) (Waters). The Waters Spherisorb column, showed greater efficiencies and better peak shapes. Therefore, this was used as a column of choice for further studies.

3.3.1.4.2 Selection of Detection Technique

Previously, Assi et al (2005) have reported the use of UV detection because it gave the maximum sensitivity without the need to introduce a derivatisation step. A wavelength (214 nm) was selected for the in-vitro analysis. When preliminary studies were carried out, a standard formoterol stock solution was prepared (50 µg/ml) in methanol and water (60:40 %v/v) and the solution was scanned from 190-400 nm using UV visible spectrophotometer to determine the optimal wavelength for the bioassay. The results showed that formoterol maximum absorbance takes place at 214 wavelength. The formoterol sample was then injected into the HPLC.

3.3.1.4.3 Selection of Mobile Phase

Initially acetonitrile: 5 mM sodium dihydrogen phosphate pH 3 (60 : 40%v/v) as determined by Assi et al (2005) was used. The percentage of acetonitrile was varied from 60 to 70 (%v/v) with a concurrent proportion of sodium dihydrogen phosphate. Since, formoterol is a weakly basic compound with a lower pH then it would be appropriate to elute the compound from the stationary phase. The pH of the buffer solution was adjusted to 3.0. The flow rate was maintained at 1.0 ml/min as reported by Assi et al (2005).

On increasing the amount of organic modifier, the retention time of formoterol decreased. The proportion of mobile phase selected helped to achieve a lower run time with formoterol at 8 minutes as shown in Figure 3.2.

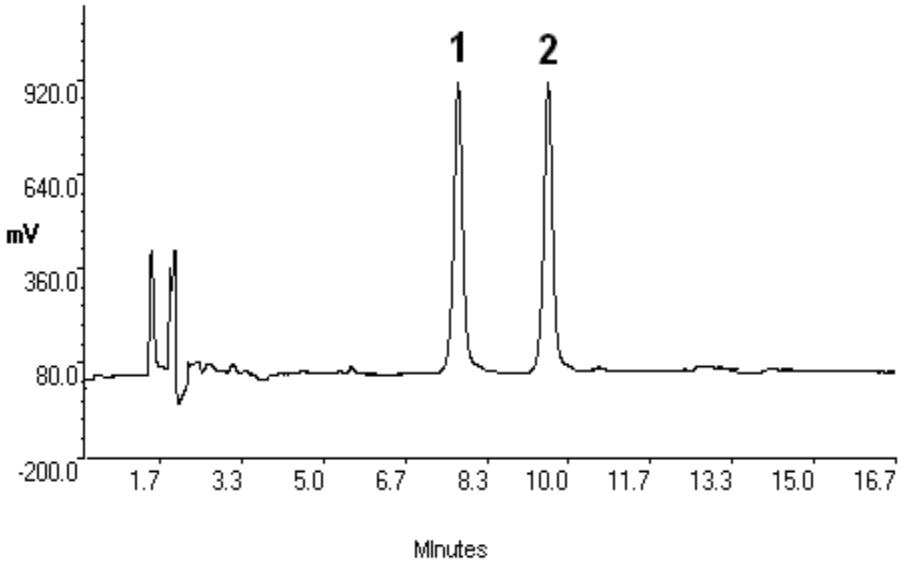

Figure 3.2: Typical Chromatogram showing the resolution of formoterol:1 and propanonol:2 (50µg/ml) injected using the following chromatography conditions: Waters Spherisorb C18 (250 mmx 4.6 mm x 5 µm); Mobile phase acetonitrile: 5 mM sodium dihydrogen phosphate, pH 3 (70:30v/v), flow rate 1 ml/min, λ=214 nm, injection volume 20 µl, column temperature 30 °C.

3.3.1.4.4 Selection of Internal Standard

A number of potential internal standards were evaluated using the conditions optimised for formoterol. The only compound of those tested, which had acceptable chromatographic behaviour, was propanolol. Propanolol is a beta-blocker that affects the heart and the circulatory system (arteries and veins). Aqueous standards of formoterol and propanolol (50µg/ml) were injected using the optimised chromatographic condition: Waters Spherisorb C18 (250 mmx 4.6 mm x 5 µm); Mobile phase acetonitrile: 5 mM sodium dihydrogen phosphate, pH 3 (70:30v/v), flow rate 1 ml/min, λ=214 nm, injection volume 20 µl, column temperature 30 °C.

3.3.4.5 Calibration Curve

In order to analyse the data obtained from the HPLC apparatus, it was necessary that an initial calibration curve was carried out using different concentrations of formoterol (50, 100, 200, 300, 400 and 500 ng/ml). The internal standard of propranolol was used so that errors were kept to a minimum in the system. The peak ratio of formoterol with the internal standard (propranolol solution) was plotted against known standard concentrations of formoterol. The amount of the drug was then calculated from the regression equation.

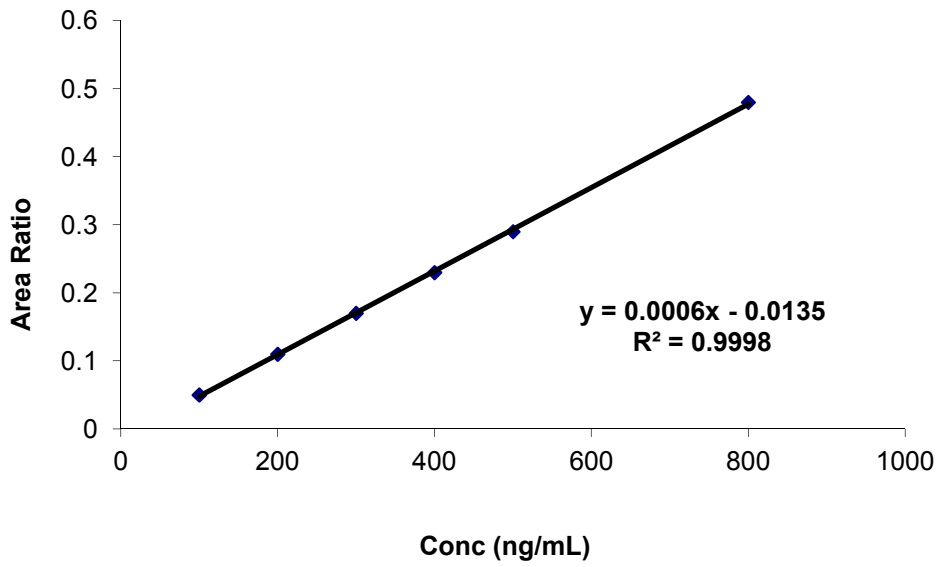

Figure 3.3: Calibration curve for formoterol aqueous standard (I.S propranolol concentration 300 ng/ml).

In summary, the conditions used in the in vitro analysis for formoterol in aqueous solutions included:

- Stationary phase (Waters Spherisorb ® C18 250 mm X 4.6 mm i.d, 5 μm column Phenomenex C18 (4 X 3 mm i.d)
- Mobile phase (70%v/v acetonitrile, 5mM, pH 3.0 and 30%v/v sodium dihydrogen phoshate)
- Flow rate (1 ml/min)
- Temperature (25˚C)
- Injection volume (100 μL)
- U.V Detection (214 nm)

CHAPTER FOUR

IN-VITRO DOSE EMISSION OF FORMOTEROL FROM THREE DPI DEVICES AT DIFFERENT INHALATION FLOWS USING LOW AND HIGH INHALATION VOLUMES

4.1 Introduction

Dry powder inhaler (DPI) research and development intensified due to the need for elimination of chlorofluocarbon (CFC) use, as a result several types of DPIs are now available (Kamin et al, 2002). All current DPIs are breath actuated, so there is an existing co-ordination between inhalation and drug released. The function of DPIs depends on air flow through the inhaler. Up to a certain level, a higher inspiratory flow through a DPI enhances drug delivery and gives better de-aggregation of the drug particles (Jaegfeldt et al, 1987) hence resulting in better drug deposition (Borgstrom et al, 1994, Newman, 1991).

DPI formulations of formoterol are available as Foradil Aeroliser® (Novartis), Oxis Turbuhaler® (AstraZeneca), and Easyhaler® (Chiese). Foradil Aeroliser contains single packed capsules while the Turbuhaler is a multidose DPI device.

The Easyhaler® device is a novel simple multidose DPI reported to be user-friendly. Previous studies have shown that the Easyhaler are therapeutically equivalent to the Turbuhaler in the administration of budesonide (Schweisfurth et al, 2002; Vanto et al, 2004).

All DPIs products are different in the formulation, the aerosol production mechanism, and the device resistance to air flow. These disparities are likely to lead to different aerosol characteristics and dose emitted. When evaluating existing or future DPI devices, the following key factors should be considered to ensure optimal dose delivery: the device's internal resistance, uniformity of the emitted dose, and aerodynamic particle size distribution, especially the respirable (fine particle) fraction of the dose. There is also a need to study the amount of drug emitted in the first inhalation and successive inhalation from a single unit dose inhaler.

In a study previously conducted by Mayer et al (2004), lung deposition and delivered dose of radiolabeled Foradil inhaled with the Aeroliser® were measured in 10 healthy

subjects. These data were then compared with data derived from an in vitro assessment of the device output and particle size distribution. It was reported that delivered dose and lung deposition significantly increased with inhalation peak flow. Flow dependant dose emission has also been reported with the Turbuhaler®. Newman et al (1991) performed a study in ten asthmatics subjects following inhalation of terbuline Turbuhaler® at inhalation flows of 57 and 28 L/min. For the higher inhalation flow, the mean (±SD) lung deposition was 16.8 (2.6) % of the nominal dose, compared to 9.1 (1.5) % at the slow inhalation flow ($p<0.001$). At either inhalation rates, the majority of the dose was deposited in the oropharynx, and quantity was significantly higher with the slow inhalation (Newman, 1991). A similar study using a budesonide Turbuhaler® confirmed the difference in lung deposition with respect to inhalation flow (Borgstrom et al, 1994). Similarly in vitro studies using Turbuhaler® containing bedesonide (Ross and Schultz, 1996) and terbutaline (Malton et al, 1996; Ross and Schultz, 1996) have revealed that dose emission is related to the inhalation flow rate. Furthermore, studies investigating the efficacy of salbutamol via Easyhaler® on pediatric and adult asthmatic patients have demonstrated less on flow rate dependency, especially at low inspiratory rates (Koskela et al, 2000).

In vitro characterisation of aerosol is useful as a pre-clinical tool to predict the amount of fine particles available upon inhalation. The present study was designed to compare the in vitro dose emission performance of three different DPI devices Foradil Aeroliser®, Oxis Turbuhaler®, and Easyhaler® to formoterol inhalation after one and two inhalations at varying flow rates. The study also compared the dose emitted at different inhalation volumes 2L and 4L.

4.2 METHODS

4.2.1 Instrumentation

4.2.1.1 Equipment and inhalation device

The various equipments used for the dose emission study are explained in Chapter 3 (Chapter 3.14).

4.2.1.2 Instrumentation set up

The dose emission method described in the compendial method (USP, 2007) for DPI was used. The sampling apparatus for dose emission enables collection of dose at high inhalation flows, up to 100 L/min (USP 2007). The mouth piece adapter ensures that there is no sample loss between the collection tube and the inhaler mouth piece. A vacuum pump with excess capacity was selected in order to achieve a desired inhalation flow. A timer controlled, two-way solenoid valve (P2 and P3) was connected between the vacuum pump and the flow controller. This valve enabled a set volume of air to be withdrawn from the mouth piece of the inhaler at a designated inhalation flow (USP, 2007). Flow control was achieved ensuring that critical (sonic) flow occurs in the flow control valve (absolute pressure ratio P3/P2 $\leq$ 0.5 under steady flow conditions).

The inhalation time was set using the relation described in the USP (2007).

T = (60 sec x X) / Q

T = Time duration consistent for withdrawal of 4 litres of air from the inhaler

Q = Airflow which produces a pressure drop of 4 kPa

X = 2 or 4 L to be drawn through the inhaler

Figure 4.1 describes the method to measure simultaneously the pressure drop across the Aeroliser® device and flow rate while measuring dose emission. The novel device was a modification of the Clark and Hollingworth (1993) method, in which absolute pressure drop across the inhaler is measured together with the mass flow. The new modified threshold enabled the determination of the pressure drop before and after the DPI to obtain an absolute pressure drop for each inhalation flow using the pressure transducer. This method measured the mass flow through the inhaler device as it is independent of temperature and atmospheric pressure changes.

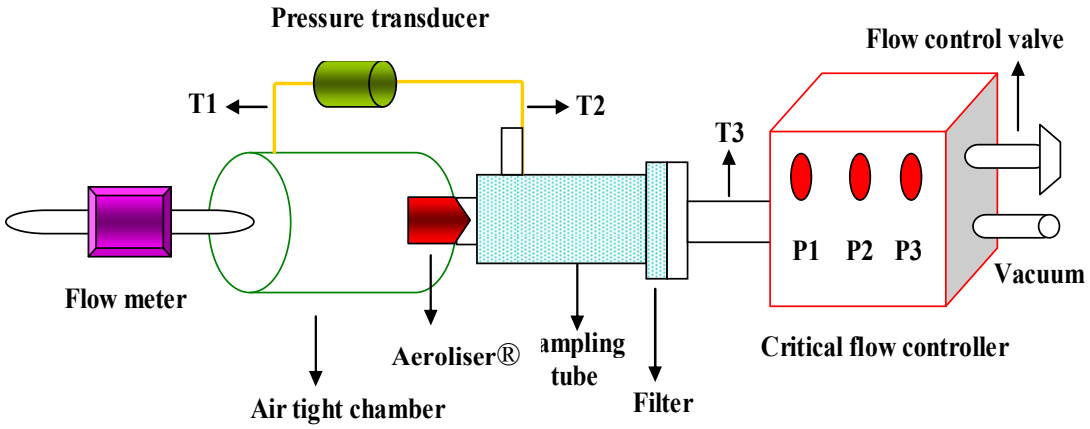

Figure 4.1: Schematic design of the method used to measure the dose emission of Foradil Aeroliser®.

4.2.2. Methodology

4.2.2.1. Dose emission measurement

The total emitted dose of formoterol from Foradil Aeroliser® containing 12 µg of formoterol per dose was determined by using sampling apparatus USP (2007) at inhalation flows of 10, 15, 20, 28.3, 60, and 90 L/min. The total dose emission of

formoterol from both Oxis Turbuhaler® and Easyhaler® containing 12 µg of formoterol per dose were determined at inhalation flows of 10, 20, 28.3, 40, and 60 L/min. The instrument was assembled as per the schematic diagram in Figure 4.1. A glass fibre filter, 47 mm (Pal Gelman Sciences, Michigan, USA) was use to collect the drug in the sampling apparatus. The unloaded inhaler device was inserted inside the chamber using a rubber mouthpiece adapter (to prevent any particle loss) and a tube T1 was connected to one end of the pressure transducer. This enabled the pressure drop across each DPI device to be determined whilst taking into consideration the atmospheric pressure and the pressure inside the sampling apparatus. The vacuum pump was switched on 20 minutes prior to each dose emission experiment in order to attain a stable flow. The two way solenoid valve opened and the desired inhalation flow was adjusted using the flow control valve. Once the flow was set, each DPI inhaler device was loaded with a dose as per instructions given in the patient information leaflet. With the vacuum pump still running and the solenoid valve closed, the inhaler was re-inserted into the mouth piece adapter horizontally. Parafilm was used in order to seal the mouth piece and the adapter to prevent any drug loss during the experimental procedure. The dose time was calculated for each inhalation flow using the equation explained in section 4.2.1.2. The powder was discharged into the sampling apparatus by activating the timer controlling the solenoid valve (USP 2007; EP 2002) so that the set inhalation volume of air was passed through the inhaler. The change in pressure drop across the inhaler was monitored simultaneously and recorded at each inhalation flow. Subsequently, 10 separate single doses for Foradil Easyhaler® and 5 separate single doses for Oxis Turbuhaler® and Easyhaler® were determined at each inhalation flow and each inhalation volume of 2 L and 4 L. After the dose was discharged into the apparatus, the inhaler device was detached and the vacuum tubing (T2) was disconnected. The collection filter was carefully removed from the sampling tube and was completely

immersed in an airtight sample bottle (to prevent evaporation of methanol) containing 20 mL of 80% methanol. The sample bottle was placed in a sonic bath for 20 minutes. This procedure was repeated two times (to ensure the entire drug entrained in the filter was released). Previously a study was performed to validate the amount of drug left over in the filter and it was performed to confirm that the entire drug leads in the filter was effectively removed during the second wash. The sampling tube was closed from one end and a 20 mL of 80% methanol was filled in the sampling tube. The other end was sealed using parafilm. The tube was shaken so as to dissolve any remaining drug particle. All the solutions were then mixed together and filtered using a membrane filter. The resultant solution was transferred into volumetric flasks and diluted to appropriate volumes prior to quantitative high performance liquid chromatography (HPLC) analysis. The HPLC analysis method is explained in section 3.3.9).

4.2.2.2 Statistical analysis

One-Way Anova was performed to determine the effect of inhalation flow rate on dose emission from Foradil Aeroliser®, Turbuhaler®, and Easyhaler®. Analysis of variance was also performed to determine the effect of different inhalation volumes of 4 L and 2 L on dose emission from these three DPI devices. The effect of one or two inhalations per single dose was also compared.

4.3 RESULTS

4.3.1 Dose emission of formoterol from a Foradil Aeroliser® at varying inhalation flows and inhalation volumes of 4 L and 2 L.

Dose emission of formoterol fumarate from a Foradil Aeroliser® was investigated at varying inhalation flows of 10, 15, 20, 28.3, 60, and 90 L/min using 4 L and 2 L inhalation volumes. The total dose emitted was also determined from 10 separate inhalations to determine device uniformity. Dose emission of formoterol from Foradil Aeroliser® was also investigated after one and two inhalations per each single dose. The results showed that the mean nominal dose emitted from Foradil Aeroliser® using one inhalation increased with increase in the inhalation flow rates ranging from 10 to 90 L/min at both 4 L (Figure 4.2 and 4.3) and 2 L (Figure 4.5 and 4.6) inhalation volumes. The mean nominal dose emission performance of Foradil Aeroliser® after second inhalation increased with increased inhalation flow rates from 10-28.3 and decreased subsequently at inhalation flow rates of 60 and 90 L/min at both 4 L (Figure 4.2 and 4.3) and 2 L (Figure 4.5 and 4.6) inhalation volumes. The capsule content decreased with increase in the inhalation flow rates from 10 to 90 L/min, both at 4 L (Figure 4.2 and 4.3) and 2 L (Figure 4.5 and 4.6) inhalation volumes. Moreover, the inhaler content also decreased with increase in the inhalation flow rates from 10 to 90 L/min at inhalation volume of 4L (Figure 4.2 and 4.3) and 2 L (Figure 4.5 and 4.6) inhalation volumes. The results from this study also showed a consistency in the total dose including emitted dose, capsule and inhaler of formoterol fumarate from Foradil Aeroliser® across a range of inhalation 10-90 L/min using inhalation volumes of 4 L (Figure 4.4) and 2 L (Figure 4.7). Comparison of mean nominal emitted dose at inhalation volumes of 4 L and 2 L demonstrated that a significantly ($p<0.001$) higher amount of drug was emitted from Foradil® Aeroliser at inhalation volume of 4 L

through varying flow rates of 10, 15, 20, 28.3, 60, and 90 L/min from both one and two inhalations at volumes compared to 2 L (Table 4.8, Figure 4.8 and 4.9). Additionally, a comparison of mean nominal dose emitted from Foradil Aeroliser® at varying flow rates shows that increase in flow rates significantly ($p<0.05$- $p<0.001$) increases amount of drug emitted at inhalation volumes of 4 L and 2 L (Table 4.7). The comparison of nominal dose emitted from Foradil Aeroliser® at varying flow rates after two inhalations showed a significant ($p<0.05$- $p<0.001$) increase in the mean nominal dose emitted at both inhalation volumes of 4 L and 2 L compared to one inhalation (Table 4.9).

In summary, mean dose emitted of formoterol from Foradil Aeroliser® increases with increase in flow rate. A more effective dose emission is achieved at inhalation volumes of 4 L. Inhaling twice after each single dose ensures a more effective emptying of the capsule and inhaler contents.

Table 4.1: Dose emitted (μg) from Foradil Aeroliser® at the different inhalation flow rates of 10 15 20 28.3 60 and 90 L/min using inhalation volume of 4 L.

Flow	**1st Inhalation**	**2nd Inhalation**	**Two inhalatios**	**Capsule**	**Inhaler**	**Total**
10 L/min						
1	5.34	1.21	6.55	2.91	2.01	11.47
2	5.29	1.29	6.58	3.23	2.45	12.26
3	5.22	1.77	6.99	3.39	1.93	12.31
4	4.89	1.91	6.80	3.78	2.61	13.19
5	5.12	1.03	6.15	3.12	2.04	11.31
6	5.89	1.05	6.94	3.45	2.91	13.30
7	5.63	1.88	7.51	3.18	2.19	12.88
8	5.12	1.71	6.83	3.24	2.57	12.64
9	5.16	1.18	6.34	2.78	2.52	11.64
10	5.19	1.13	6.32	2.17	2.15	10.64
Mean	5.29	1.42	6.70	3.13	2.34	12.16
S.D.	0.28	0.36	0.40	0.44	0.32	0.88
%	44.04	11.80	55.84	26.04	19.48	101.37

15 L/min

1	6.13	1.33	7.46	2.36	1.45	11.27
2	6.04	2.06	8.10	2.59	1.93	12.62
3	5.99	2.12	8.11	2.92	1.99	13.02
4	5.53	1.98	7.51	2.01	2.01	11.53
5	5.99	1.91	7.90	2.19	1.29	11.38
6	5.34	1.21	6.55	2.46	1.39	10.40
7	5.94	1.32	7.26	2.34	1.74	11.34
8	6.13	2.02	8.15	2.75	1.81	12.71
9	6.26	2.25	8.51	2.71	1.91	13.13
10	5.99	1.19	7.18	2.11	1.22	10.51
Mean	5.93	1.74	7.67	2.44	1.67	11.79
S.D.	0.28	0.42	0.59	0.30	0.31	1.01
%	49.45	14.49	63.91	20.37	13.95	98.26

20 L/min

1	7.11	2.33	9.44	1.33	1.23	12.00
2	7.34	2.76	10.10	1.38	1.66	13.14
3	6.97	3.12	10.09	2.12	1.05	13.26
4	6.23	2.78	9.01	2.09	1.25	12.35
5	7.99	2.01	10.00	0.77	1.29	12.06
6	7.44	2.11	9.55	1.09	2.03	12.67
7	6.34	1.92	8.26	1.67	1.93	11.86
8	7.03	3.02	10.05	1.29	0.91	12.25
9	7.56	2.22	9.78	0.92	0.99	11.69
10	7.29	2.45	9.74	1.33	1.78	12.85
Mean	7.13	2.47	9.60	1.40	1.41	12.41
S.D.	0.53	0.43	0.58	0.45	0.41	0.54
%	59.42	20.60	80.00	11.66	11.77	103.44

28.3 L/min

1	9.23	1.81	11.04	0.34	0.23	11.61
2	8.89	3.65	12.54	0.74	0.11	13.39
3	8.54	3.39	11.93	0.77	0.13	12.83
4	9.04	3.19	12.23	0.45	0.10	12.78
5	10.55	1.23	11.78	0.65	0.05	12.48
6	7.56	4.03	11.59	0.98	0.10	12.67
7	8.34	1.98	10.32	0.94	0.21	11.47
8	7.98	4.06	12.04	1.04	0.32	13.40
9	8.61	1.73	10.34	0.66	0.13	11.13

10	9.02	2.00	11.02	0.51	0.04	11.57
Mean	8.78	2.71	11.48	0.71	0.14	12.33
S.D.	0.81	1.06	0.77	0.23	0.09	0.83
%	73.13	22.56	95.66	5.90	1.18	102.78

60 L/min

1	10.22	1.93	12.15	0.54	0.10	12.79
2	9.56	1.74	11.30	0.53	0.15	11.98
3	9.34	1.77	11.11	0.23	0.14	11.48
4	10.12	1.91	12.03	0.57	0.12	12.72
5	10.02	1.18	11.20	0.38	0.01	11.59
6	9.87	1.31	11.18	0.59	0.32	12.09
7	9.69	1.88	11.57	0.28	0.05	11.90
8	9.61	1.71	11.32	0.34	0.15	11.81
9	8.82	1.85	10.67	0.66	0.23	11.56
10	9.91	1.47	11.38	0.59	0.07	12.04
Mean	9.72	1.68	11.39	0.47	0.13	12.00
S.D.	0.42	0.26	0.44	0.15	0.09	0.45
%	80.97	13.96	94.91	3.93	1.12	99.97

90 L/min

1	10.76	1.03	11.79	0.39	0.00	12.18
2	10.65	0.87	11.52	0.26	0.00	11.78
3	10.12	1.11	11.23	0.29	0.01	11.53
4	9.34	0.78	10.12	0.31	0.03	10.46
5	11.06	0.55	11.61	0.38	0.00	11.99
6	11.34	1.04	12.38	0.18	0.00	12.56
7	10.35	1.28	11.63	0.46	0.07	12.16
8	10.98	0.52	11.50	0.21	0.00	11.71
9	10.77	1.07	11.84	0.47	0.03	12.34
10	10.34	0.99	11.33	0.34	0.00	11.67
Mean	10.57	0.92	11.50	0.33	0.01	11.84
S.D.	0.57	0.24	0.58	0.10	0.02	0.58
%	88.09	7.70	95.83	2.74	0.12	98.65

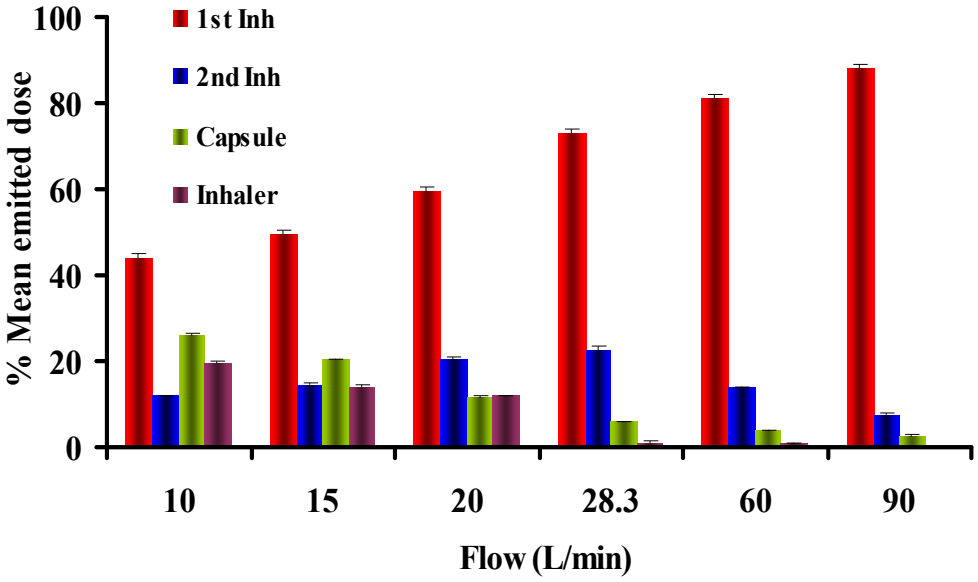

Figure 4.2: Mean emitted dose (%) from first and second inhalations, capsule, and inhaler of formoterol fumarate from a Foradil Aeroliser® at varying inhalation flow rates (10-90 L/min) using inhalation volume of 4 L.

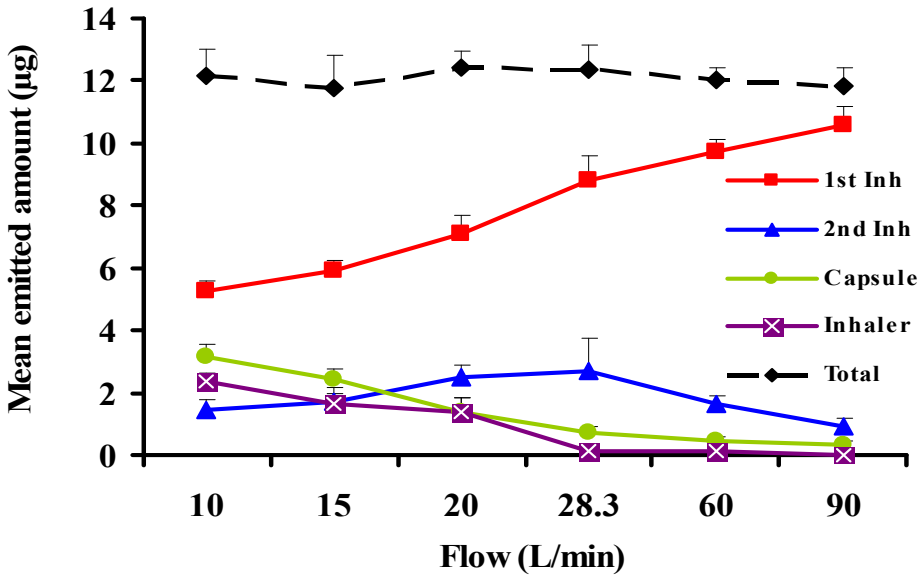

Figure 4.3: Mean emitted dose (µg) from first and second inhalations, capsule, and inhaler of formoterol fumarate from a Foradil Aeroliser® at varying inhalation flow rates (10-90 L/min) using inhalation volume of 4 L.

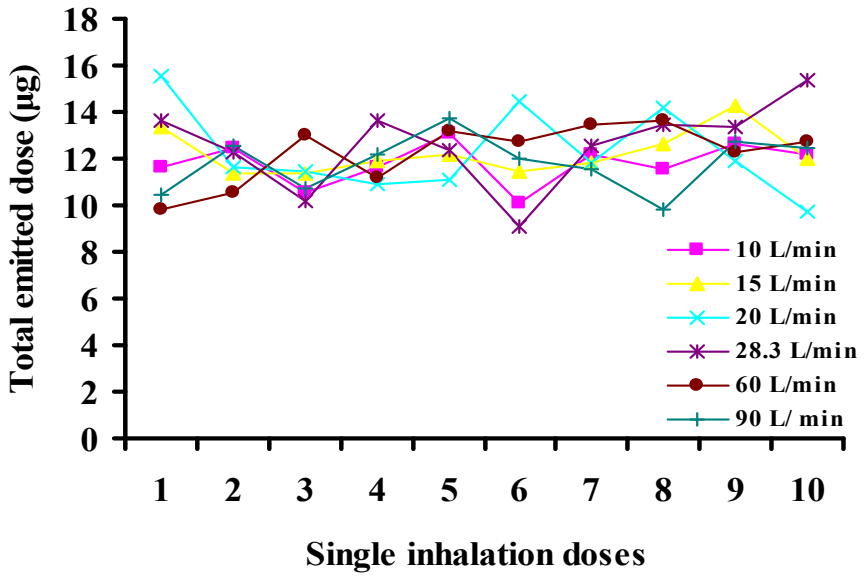

Figure 4.4: Total dose (µg) of a Foradil Aeroliser® including emitted dose, capsule and inhaler from 10 separate single inhalation doses at different inhalation flow rates of 10 15 20 28.3 60 and 90 L/min using inhalation volumes of 4 L.

Table 4.2: Dose emitted (µg) Foradil Aeroliser® at the different inhalation flow rates of 10, 15, 20, 28.3, 60 and 90 L/min at inhalation volume of 2 L.

Flows	**1st Inhalation**	**2nd Inhalation**	**Two inhalations**	**Capsule**	**Inhaler**	**Total**
10 L/min						
1	2.91	0.78	3.69	6.23	1.73	11.65
2	2.77	0.98	3.75	6.78	1.93	12.46
3	2.59	1.05	3.64	5.91	1.01	10.56
4	1.58	2.13	3.71	7.01	0.94	11.66
5	3.99	0.94	4.93	5.99	2.14	13.06
6	2.89	0.57	3.46	4.89	1.73	10.08
7	3.45	0.68	4.13	6.93	1.09	12.15
8	2.84	0.45	3.29	5.12	2.03	11.55
9	3.95	0.93	4.88	6.85	1.99	12.61
10	2.33	1.06	3.39	6.90	1.89	12.18
Mean	2.93	0.96	3.89	6.26	1.65	11.80
S.D.	0.73	0.46	0.58	0.78	0.46	0.92
%	24.42	7.98	32.41	52.18	13.73	98.30

15 L/min

1	2.31	0.73	3.04	8.41	1.93	13.38
2	1.21	0.90	2.11	6.91	2.34	11.36
3	2.01	0.23	2.24	7.01	2.12	11.37
4	3.02	0.66	3.68	6.12	2.10	11.90
5	2.16	1.13	3.29	7.12	1.78	12.19
6	2.69	1.09	3.78	5.56	2.07	11.41
7	2.37	1.72	4.09	6.34	1.38	11.81
8	1.43	0.99	2.42	7.66	2.53	12.61
9	2.19	0.29	2.48	8.89	2.91	14.28
10	1.77	0.62	2.39	8.32	1.33	12.04
Mean	2.12	0.84	2.95	7.23	2.05	12.24
S.D.	0.55	0.44	0.72	1.08	0.48	0.95
%	17.63	6.97	24.58	60.28	17.08	101.96

20 L/min

1	4.99	2.13	7.12	6.78	1.65	15.55
2	4.12	1.34	5.46	4.31	1.85	11.62
3	2.93	0.93	3.86	5.56	2.04	11.46
4	2.78	1.12	3.90	5.98	0.99	10.87
5	2.94	2.47	5.41	5.09	0.57	11.07
6	4.09	3.73	7.82	5.38	1.29	14.49
7	3.94	1.34	5.28	4.93	1.62	11.83
8	3.71	1.56	5.27	6.79	2.11	14.17
9	2.96	1.21	4.17	6.39	1.34	11.90
10	2.87	1.61	4.48	4.03	1.19	9.70
Mean	3.53	1.74	5.28	5.52	1.47	12.27
S.D.	0.75	0.84	1.32	0.97	0.48	1.85
%	29.44	14.53	44.00	46.03	12.21	102.22

28.3 L/min

1	5.32	1.81	7.13	4.93	1.56	13.62
2	4.55	2.61	7.16	4.34	0.73	12.23
3	5.02	1.34	6.36	3.09	0.73	10.18
4	5.12	1.92	7.04	4.94	1.63	13.61
5	5.18	2.01	7.19	4.42	0.77	12.38
6	3.09	1.13	4.22	3.91	0.98	9.11
7	4.78	0.99	5.77	5.09	1.70	12.56
8	3.81	2.23	6.04	6.18	1.23	13.45
9	4.19	1.98	6.17	6.56	0.60	13.33

10	5.58	2.56	8.14	5.87	1.39	15.40
Mean	4.66	1.86	6.52	4.93	1.13	12.59
S.D.	0.77	0.56	1.07	1.06	0.42	1.81
%	38.87	15.48	54.33	41.11	9.43	104.89

60 L/min

1	5.34	0.45	5.79	2.56	1.45	9.80
2	5.23	0.98	6.21	3.67	0.65	10.53
3	5.91	1.23	7.14	4.79	1.06	12.99
4	4.92	1.34	6.26	4.34	0.56	11.16
5	5.45	1.51	6.96	5.09	1.17	13.22
6	5.69	0.94	6.63	5.07	1.06	12.76
7	5.09	0.96	6.05	6.66	0.73	13.44
8	6.37	1.48	7.85	4.56	1.23	13.64
9	5.93	0.78	6.71	3.56	2.02	12.29
10	5.12	1.50	6.62	5.16	0.92	12.70
Mean	5.51	1.12	6.62	4.55	1.09	12.25
S.D.	0.46	0.35	0.60	1.12	0.43	1.31
%	45.88	9.30	55.16	37.88	9.04	102.10

90 L/min

1	5.23	0.77	6.00	3.03	1.45	10.48
2	6.34	0.54	6.88	3.99	1.65	12.52
3	5.76	1.05	6.81	3.12	0.78	10.71
4	6.34	1.34	7.68	3.98	0.56	12.22
5	6.83	1.67	8.50	4.29	0.97	13.76
6	6.03	0.65	6.68	4.12	1.16	11.96
7	5.12	0.77	5.89	4.92	0.73	11.54
8	4.15	0.45	4.60	3.95	1.23	9.78
9	6.03	0.87	6.90	4.78	1.02	12.70
10	5.63	0.65	6.28	5.30	0.92	12.50
Mean	5.75	0.88	6.62	4.15	1.05	11.82
S.D.	0.76	0.38	1.05	0.73	0.33	1.20
%	47.88	7.30	55.16	34.57	8.73	98.48

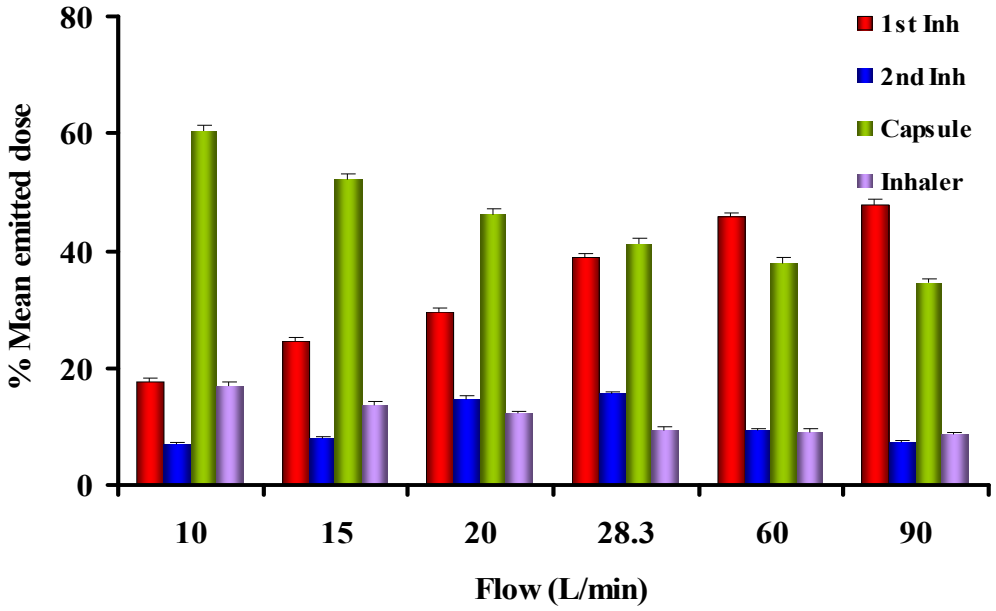

Figure 4.5: Mean emitted dose (%) from first and second inhalations, capsule, and inhaler of formoterol fumarate from a Foradil Aeroliser® at varying inhalation flow rates (10-90 L/min) using inhalation volume of 2 L.

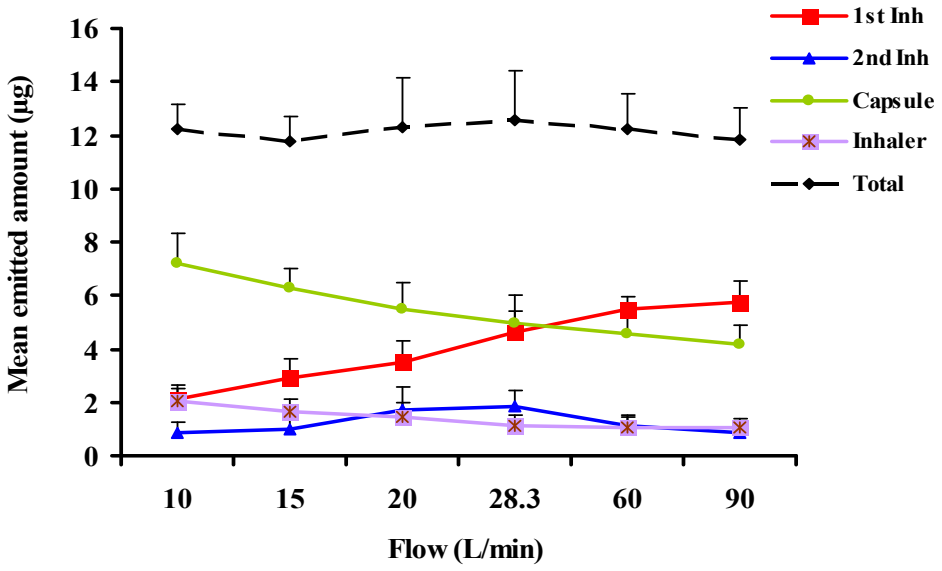

Figure 4.6: Mean emitted dose (µg) from first and second inhalations, capsule, and inhaler of formoterol fumarate from a Foradil Aeroliser® at varying inhalation flow rates (10-90 L/min) using inhalation volumes of 2 L.

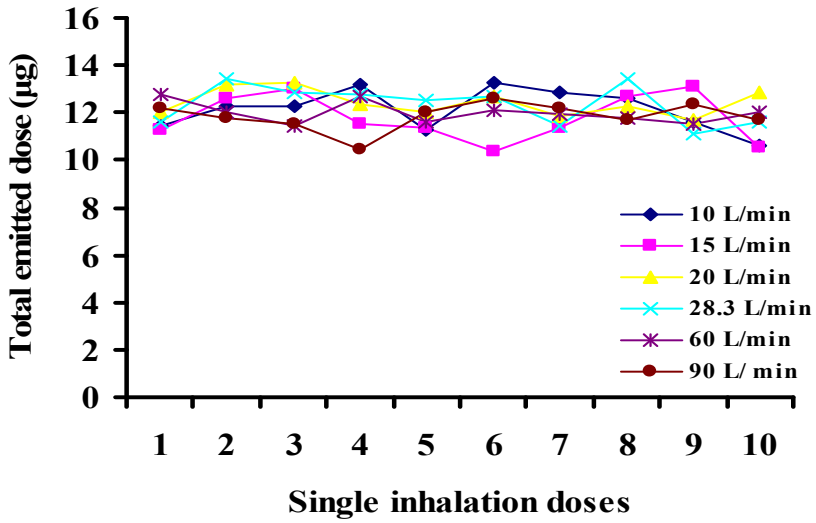

Figure 4.7:Total dose (µg) of a Foradil Aeroliser® from10 separate single inhalation doses at different inhalation flow rates of 10 15 20 28.3 60 and 90 L/min and inhalation volumes of 2 L.

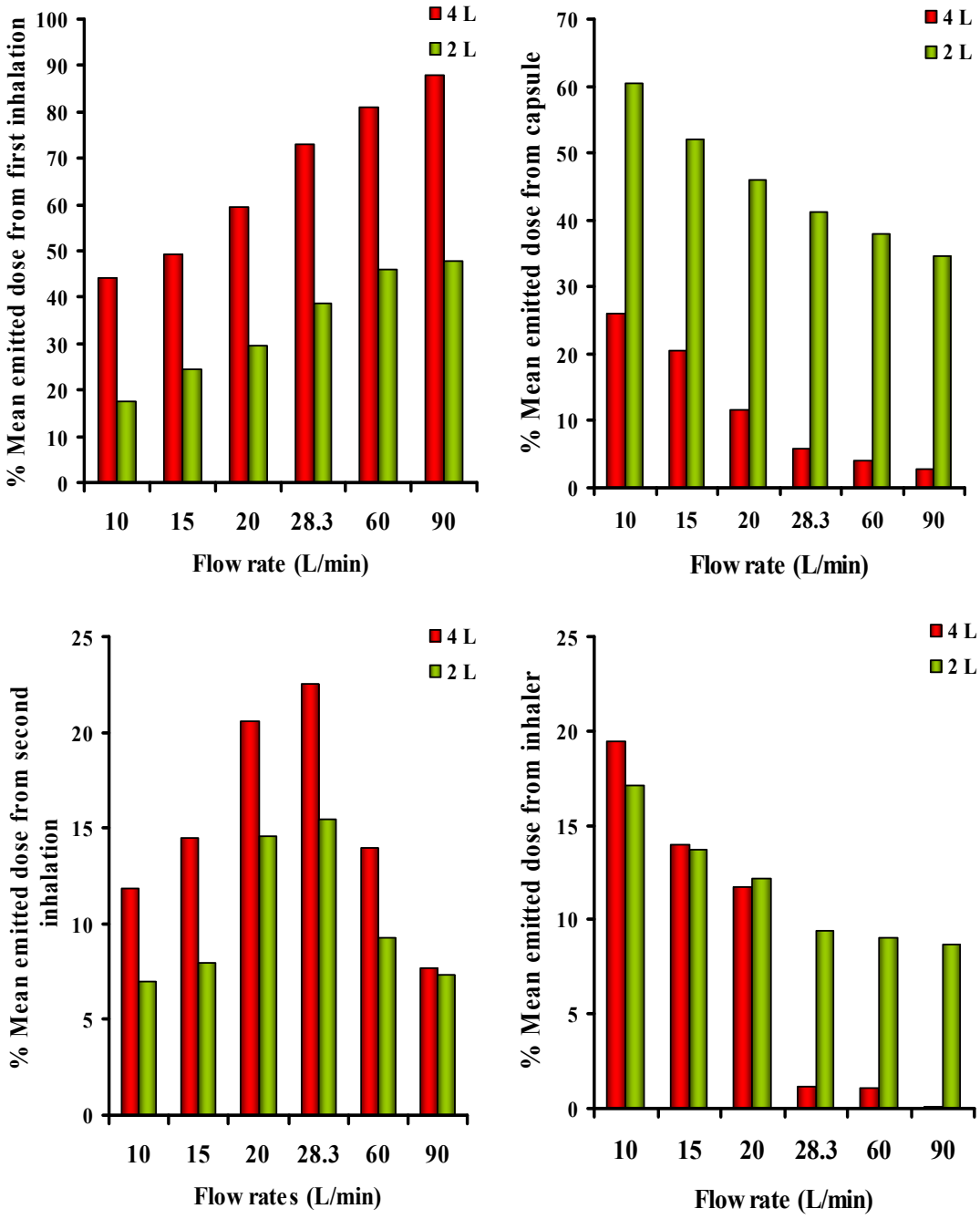

Figure 4.8: Comparison of mean emitted dose (%) from first and second inhalations, capsule, and inhaler of formoterol fumarate from a Foradil Aeroliser® at varying inhalation flow rates (10-90 L/min) using inhalation volumes of 4 L and 2 L.

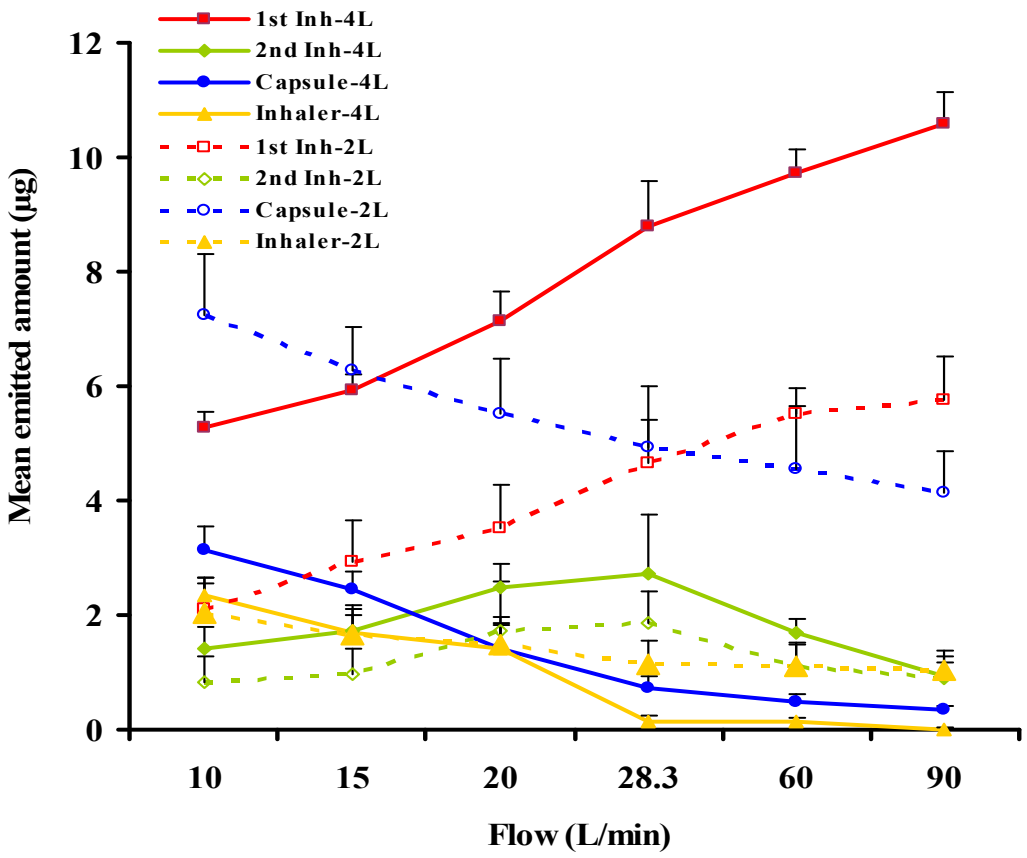

Figure 4.9: Comparison of mean emitted dose (µg) from first and second inhalations, capsule, and inhaler of formoterol fumarate from a Foradil Aeroliser® at varying inhalation flow rates (10-90 L/min) using inhalation volumes of 4 L and 2 L.

4.3.2 Dose emission of formoterol from an Oxis Turbuhaler® at varying inhalation flows using inhalation volumes of 4 L and 2 L.

Dose emission of formoterol fumarate from Oxis Turbuhaler® was investigated at varying inhalation flows of 10, 20, 28.3, 40, and 60 L/min using 4 L and 2 L inhalation volumes. The total dose emitted was also determined from five separate inhalations to determine device uniformity. Dose emission of formoterol from Oxis Turbuhaler® was also investigated after one and two inhalations per each single dose. The results showed that the mean nominal dose emitted from Oxis Turbuhaler® after one inhalation increased with increase in the inhalation flow rates ranging from 10 to 60 L/min at both 4 L (Figure 4.10 and 4.11) and 2 L (Figure 4.13 and 4.14) inhalation volumes. Also, the mean nominal dose emission performance of Oxis Turbuhaler® after two inhalations increased with increased inhalation flow rates at both 4 L (Figure 4.10 and 4.11) and 2 L (Figure 4.13 and 4.14) inhalation volumes. Comparison of mean nominal emitted dose at inhalation volumes of 4 L and 2 L demonstrated that a significantly ($p<0.001$) higher amount of drug was emitted from Oxis Turbuhaler® at inhalation volume of 4 L through varying flow rates of 10, 20, 28.3, 40, and 60 L/min compared to 2 L (Table 4.11 and Figure 4.16). Additionally, a comparison of mean nominal dose emitted from Oxis Turbuhaler® at varying flow rates shows that increase in flow rates from 10 to 40 L/min significantly ($p<0.05$- $p<0.001$) increases amount of drug emitted at inhalation volumes of 4 L and 2 L but not at inhalation flow rate >40 L/min (Table 4.10). The comparison of nominal dose emitted from Oxis Turbuhaler® at varying flow rates, two inhalations showed a significant ($p<0.05$- $p<0.01$) increase in the mean nominal dose emitted at inhalation volumes of 2 L compared to one inhalation but not at inhalation volumes of 4 L (Table 4.12). In summary, mean dose emitted of formoterol from Oxis Turbuhaler® increases with increase in flow rate especially at lower flow rates. A more effective dose emission is

achieved at inhalation volumes of 4 L. Inhaling twice after each single dose ensures a more effective dose emission only at inhalation volumes of 2 L suggesting that those suffering from lung disorders will be able to achieve a better formoterol dose emission by inhaling twice from an Oxis Turbuhaler®.

Table 4.3: Dose emitted (μg) from Oxis Turbuhaler® at the different inhalation flow rates of 10, 20, 28.3, 40 and 60 L/min using inhalation volume of 4 L.

Flows	**One Inhalation**	**Two Inhalations**
10 L/min		
1	5.22	5.36
2	5.35	5.50
3	5.31	5.46
4	5.28	5.42
5	5.26	5.40
Mean	5.28	5.43
S.D.	0.05	0.05
%	44.04	45.23
20 L/min		
1	6.76	6.99
2	7.01	7.26
3	6.87	7.01
4	6.99	7.07
5	6.83	6.93
Mean	6.89	7.05
S.D.	0.11	0.13
%	57.44	58.76
28.3 L/min		
1	7.50	7.72
2	7.68	7.92
3	7.49	7.73
4	7.95	8.19
5	7.58	7.80
Mean	7.64	7.87
S.D.	0.19	0.19
%	63.66	65.60

40 L/min		
1	8.32	9.01
2	7.91	8.56
3	7.96	8.59
4	8.04	8.71
5	8.01	8.67
Mean	8.05	8.71
S.D.	0.16	0.18
%	67.07	69.84

60 L/min		
1	9.00	9.63
2	8.52	8.98
3	8.45	8.21
4	8.73	9.30
5	9.19	9.15
Mean	8.78	9.05
S.D.	0.31	0.53
%	73.16	75.46

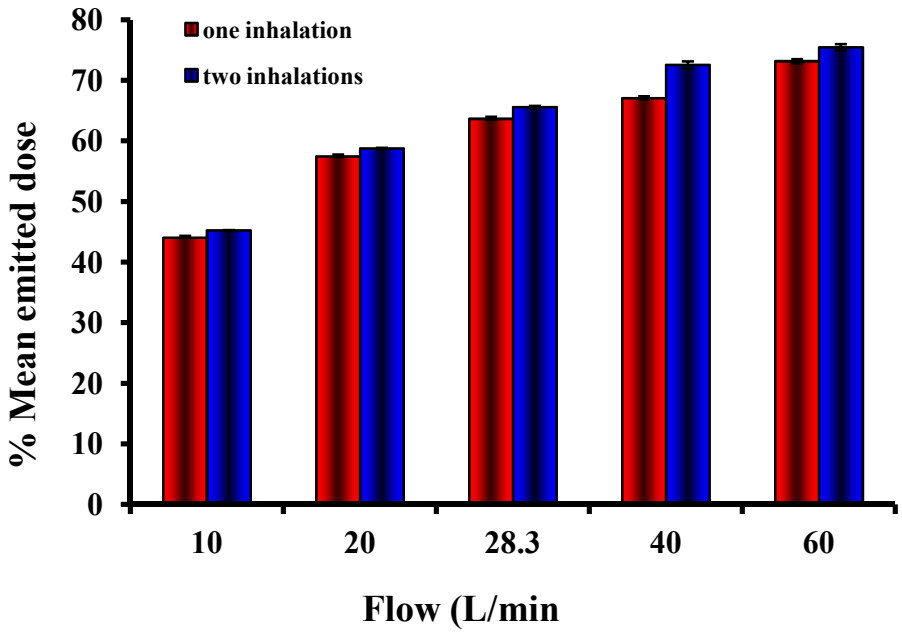

Figure 4.10: Mean emitted dose (%) from one and two inhalations of formoterol fumarate from Oxis Turbuhaler® at varying inhalation flow rates (10-60 L/min) using inhalation volume of 4 L.

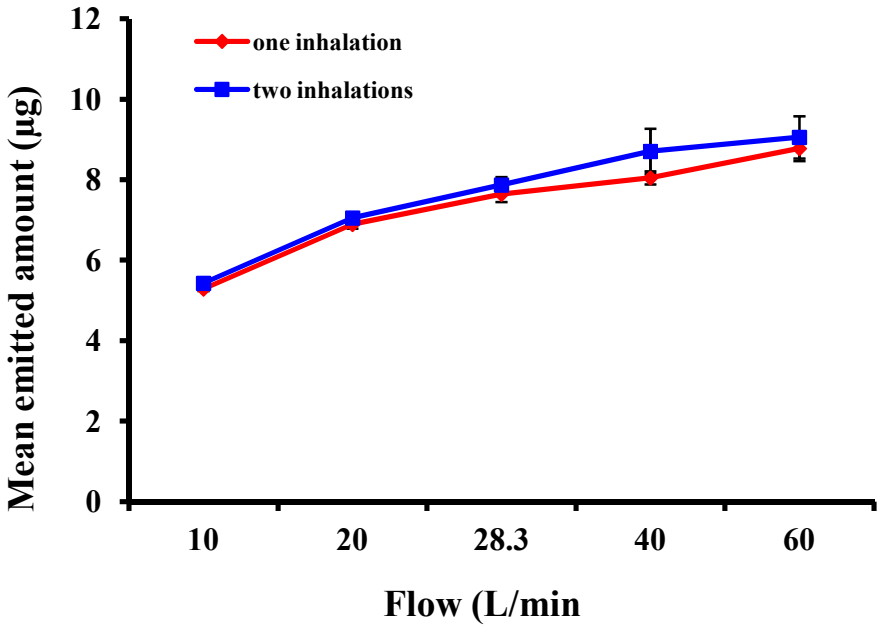

Figure 4.11: Mean emitted dose (µg) from one and two inhalations of formoterol fumarate from a Oxis Turbuhaler® at varying inhalation flow rates (10-60 L/min) using inhalation volume of 4 L.

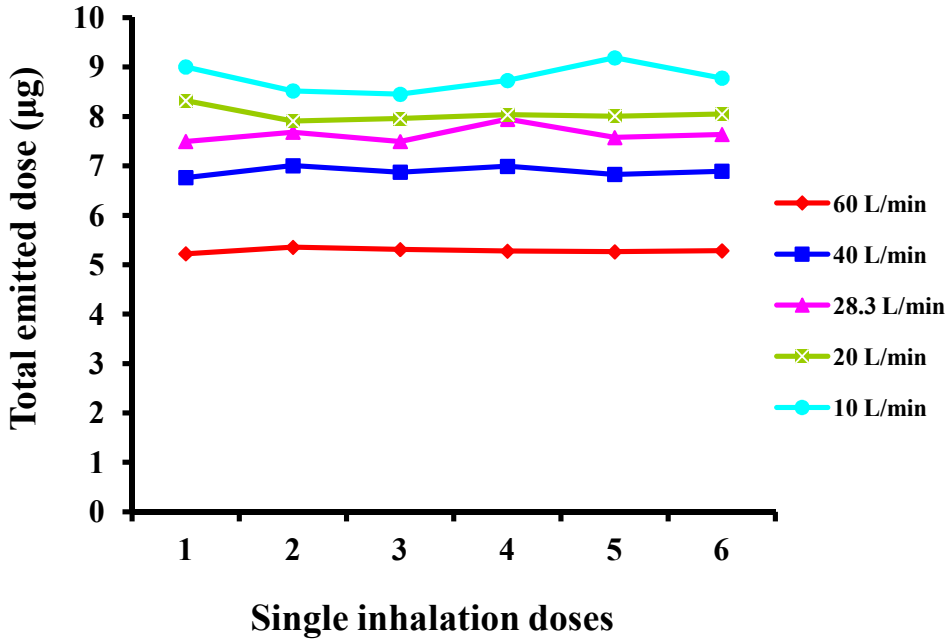

Figure 4.12: Total dose emitted (µg) of 5 separate single inhalation doses of formoterol fumarate from an Oxis Turbuhaler® at different inhalation flow rates of 10, 20, 28.3, 40, and 60 L/min and inhalation volumes of 4 L.

Table 4.4: Dose emitted (µg) Oxis Turbuhaler® at the different inhalation flow rates of 10, 20, 28.3, 40 and 60 L/min at inhalation volume of 2 L.

Flows	One inhalation	Two inhalations
10 L/min		
1	3.70	3.72
2	3.86	3.86
3	4.00	3.98
4	3.84	3.84
5	3.66	3.68
Mean	3.81	3.82
S.D.	0.14	0.12
%	31.77	31.80
20 L/min		
1	5.45	5.83
2	5.67	6.05
3	5.80	6.18
4	5.65	6.03
5	5.43	5.81
Mean	5.60	5.98
S.D.	0.15	0.16
%	46.66	49.81
28.3 L/min		
1	6.15	6.59
2	6.31	6.76
3	6.36	6.80
4	6.30	6.75
5	6.15	6.59
Mean	6.25	6.70
S.D.	0.10	0.10
%	52.16	55.82

40 L/min		
1	6.34	6.80
2	6.47	6.94
3	6.49	6.94
4	6.45	6.92
5	6.29	6.75
Mean	6.41	6.87
S.D.	0.09	0.09
%	53.38	57.25

60 L/min		
1	6.65	7.15
2	6.73	7.23
3	6.74	7.23
4	6.71	7.20
5	6.47	6.96
Mean	6.66	7.15
S.D.	0.11	0.11
%	55.51	59.62

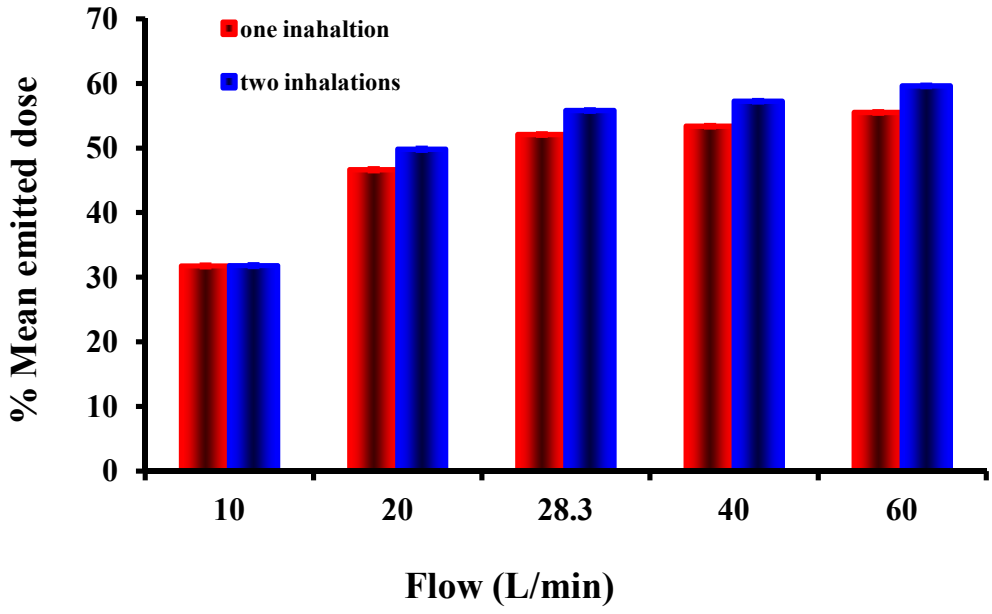

Figure 4.13: Mean emitted dose (%) from one and two inhalations of formoterol fumarate from Oxis Turbuhaler® at varying inhalation flow rates (10-60 L/min) using inhalation volume of 2 L.

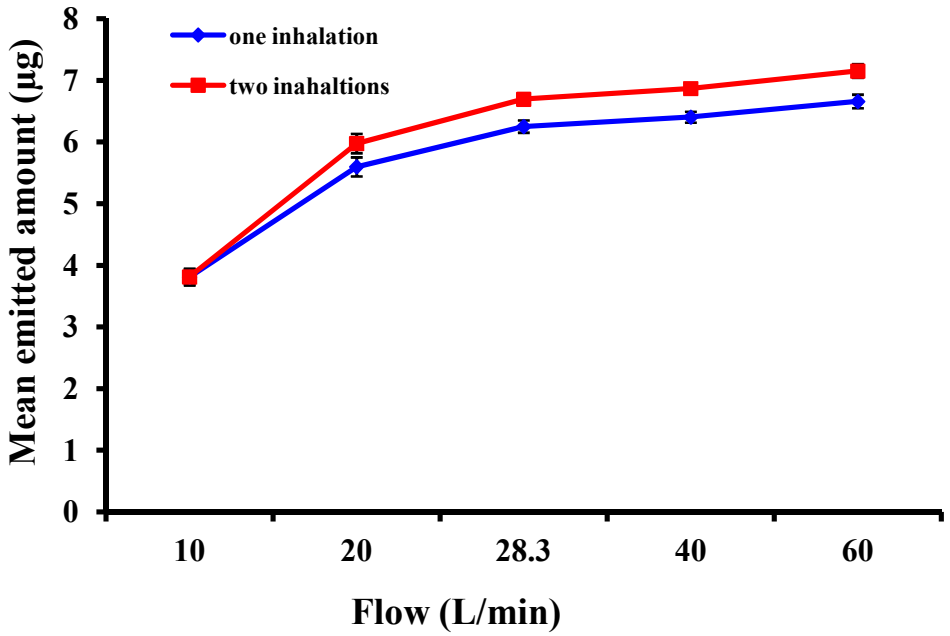

Figure 4.14: Mean emitted dose (µg) from one and two inhalations of formoterol fumarate from an Oxis Turbuhaler® at varying inhalation flow rates (10-60 L/min) using inhalation volume of 2 L.

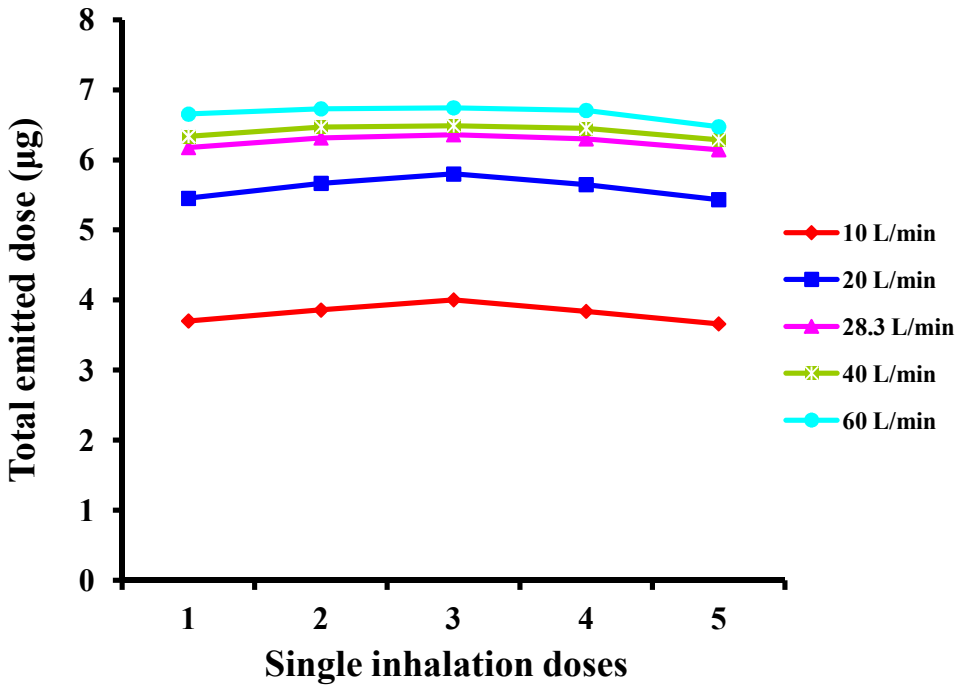

Figure 4.15: Total dose emitted (µg) of 5 separate single inhalation doses of formoterol fumarate from an Oxis Turbuhaler® at different inhalation flow rates of 10, 20, 28.3, 40, and 60 L/min and inhalation volumes of 2 L.

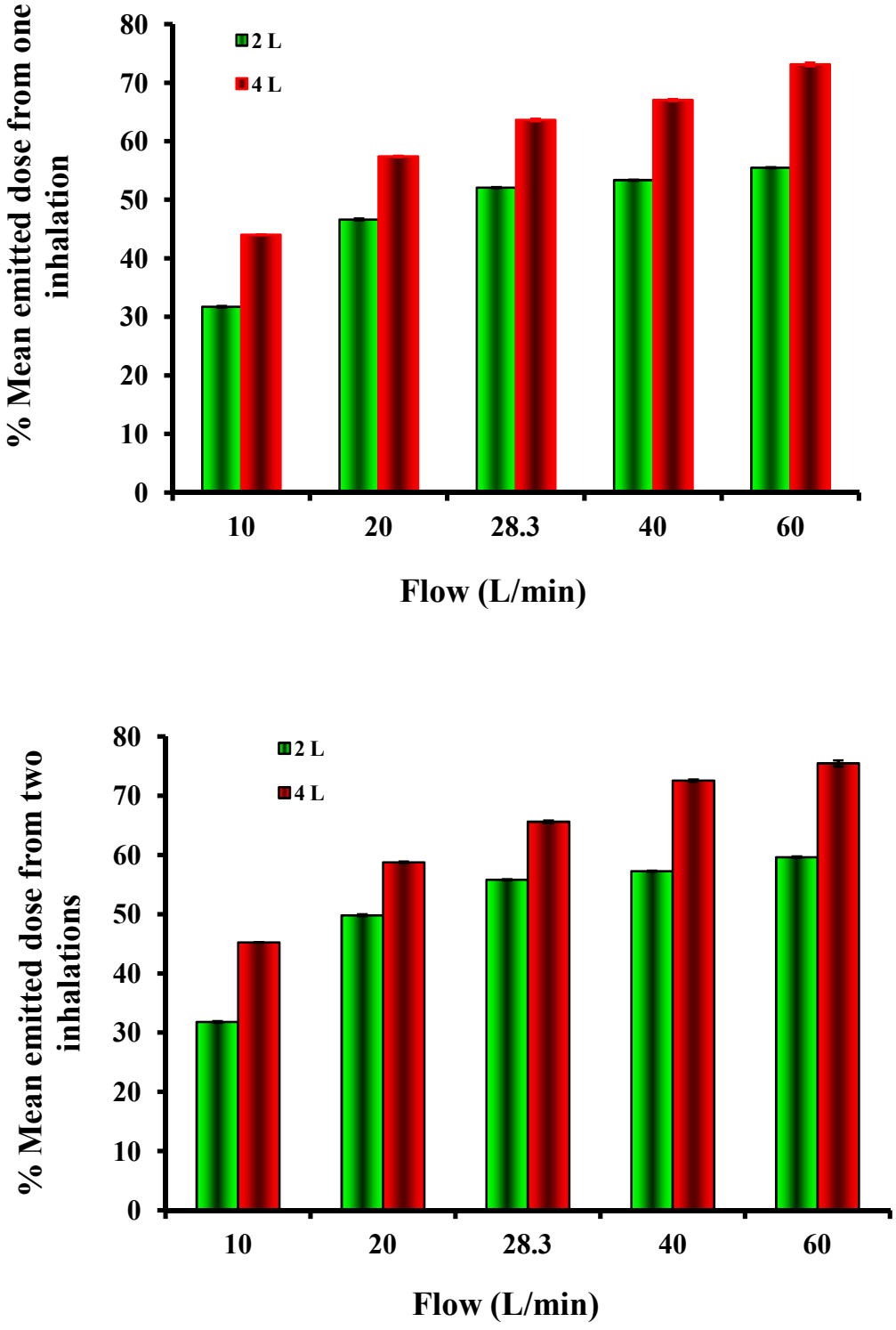

Figure 4.16: Comparison of mean emitted dose (%) from one and two inhalations of formoterol fumarate from an Oxis Turbuhaler® at varying inhalation flow rates (10-60 L/min) using inhalation volumes of 4 L and 2 L.

4.3.3 Dose emission of formoterol from Easyhaler® at varying inhalation flows using inhalation volumes of 4 L and 2 L.

Dose emission of formoterol fumarate from an Easyhaler® was investigated at varying inhalation flows of 10, 20, 28.3, 40, and 60 L/min using 4 L and 2 L inhalation volumes. The total dose emitted was also determined from five separate inhalations and after one and two inhalations. The results showed that the mean nominal dose emitted from Easyhaler® using one inhalation increased with increase in the inhalation flow rates ranging from 10 to 60 L/min at both 4 L (Figure 4.17 and 4.18) and 2 L (Figure 4.20 and 4.21) inhalation volumes. Also, the mean nominal dose emission performance of Easyhaler® from two inhalations increased with increasing inhalation flow rates at both 4 L (Figure4.17 and 4.18) and 2 L (Figure 4.20 and 4.21) inhalation volumes. Comparison of mean nominal emitted dose at inhalation volumes of 4 L and 2 L demonstrated that a significantly ($p<0.001$) higher amount of the drug was emitted from Easyhaler® at inhalation volume of 4 L through varying flow rates of 10, 20, 28.3, 40, and 60 L/min compared to 2 L (Table 4.14, Figure 4.23). Additionally, a comparison of mean nominal dose emitted from Easyhaler® at varying flow rates shows that increase in flow rates significantly ($p<0.05$- $p<0.001$) increases amount of the drug emitted at inhalation volumes of 4 L and 2 L (Table 4.13). Furthermore, the comparison of nominal dose emitted from Easyhaler® after two inhalations showed significant ($p<0.05$) increase at inhalation flow rates of 40 and 60 L/min using 2 L and 4 L inhalation volumes compared to one inhalation. Interestingly, inhalation flow rates of 10, 20, and 28.3 L/min showed no significant differences (Table 4.15). In summary, mean dose emitted of formoterol from Easyhaler® increases with increase in flow rate especially at lower inhalation flow rates. A more effective dose emission is achieved at inhalation volumes of 4 L. Inhaling twice after each single dose ensures a more effective dose emission at higher inhalation flow.

Table 4.5: Dose emitted (μg) from Easyhaler® at the different inhalation flow rates of 10, 20, 28.3, 40 and 60 L/min using inhalation volume of 4 L.

Flows	**One inhalation**	**Two inhalations**
10 L/min		
1	7.14	7.27
2	6.77	7.80
3	6.90	7.61
4	7.98	7.86
5	7.60	7.14
Mean	7.28	7.54
S.D.	0.50	0.32
%	60.63	62.80
20 L/min		
1	8.32	8.91
2	8.70	9.53
3	8.47	8.21
4	8.90	8.64
5	8.59	8.47
Mean	8.60	8.75
S.D.	0.22	0.50
%	71.63	72.93
28.3 L/min		
1	9.63	10.15
2	9.91	10.94
3	10.79	10.17
4	10.38	10.38
5	10.52	11.12
Mean	10.25	10.55
S.D.	0.47	0.45
%	85.38	87.92
40 L/min		
1	10.364	10.772
2	10.488	10.704
3	10.719	11.013
4	10.551	10.812
5	10.616	10.884
Mean	10.5476	10.837

S.D.	0.133538	0.11803
%	87.89667	90.31

60 L/min

1	10.94	11.49
2	10.46	11.34
3	10.81	11.76
4	10.43	11.20
5	11.45	10.98
Mean	10.82	11.35
S.D.	0.42	0.29
%	90.15	94.62

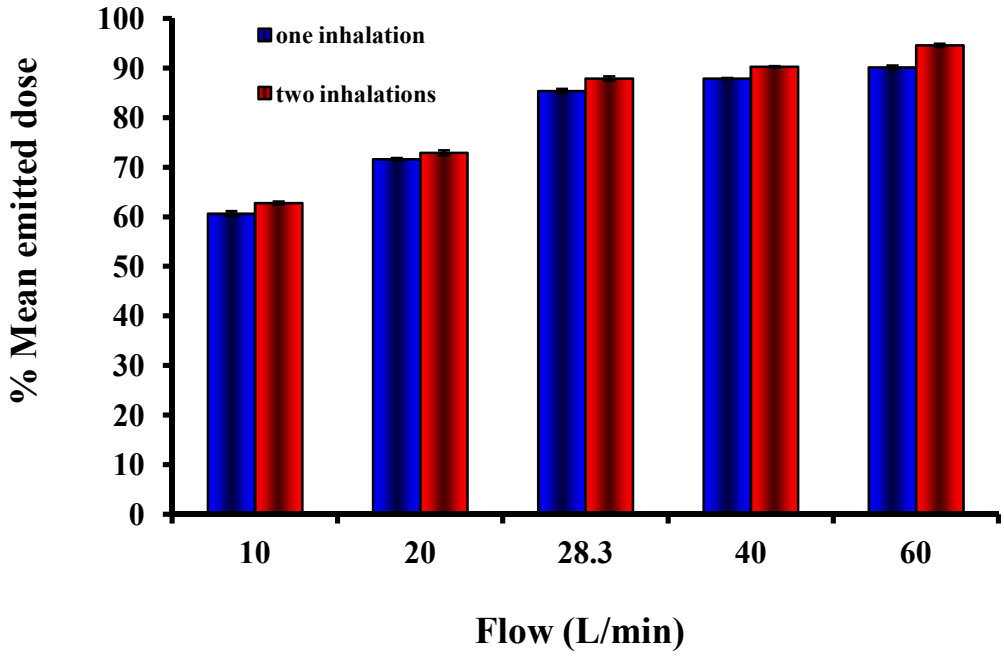

Figure 4.17: Mean emitted dose (%) from one and two inhalations of formoterol fumarate from Easyhaler® at varying inhalation flow rates (10-60 L/min) using inhalation volume of 4 L.

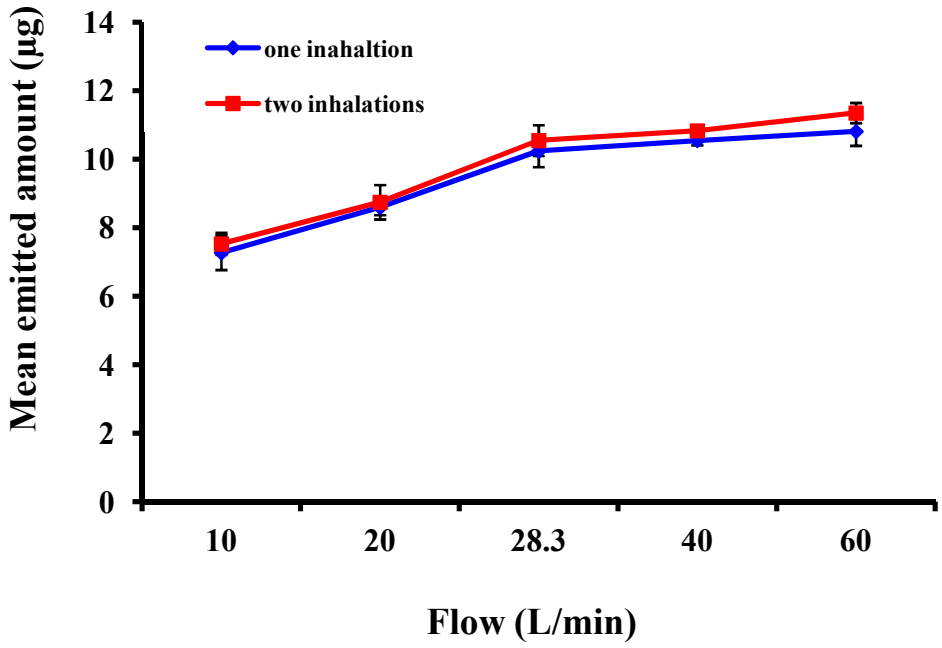

Figure 4.18: Mean emitted dose (µg) from one and two inhalations of formoterol fumarate from an Easyhaler® at varying inhalation flow rates (10-60 L/min) using inhalation volume of 4 L.

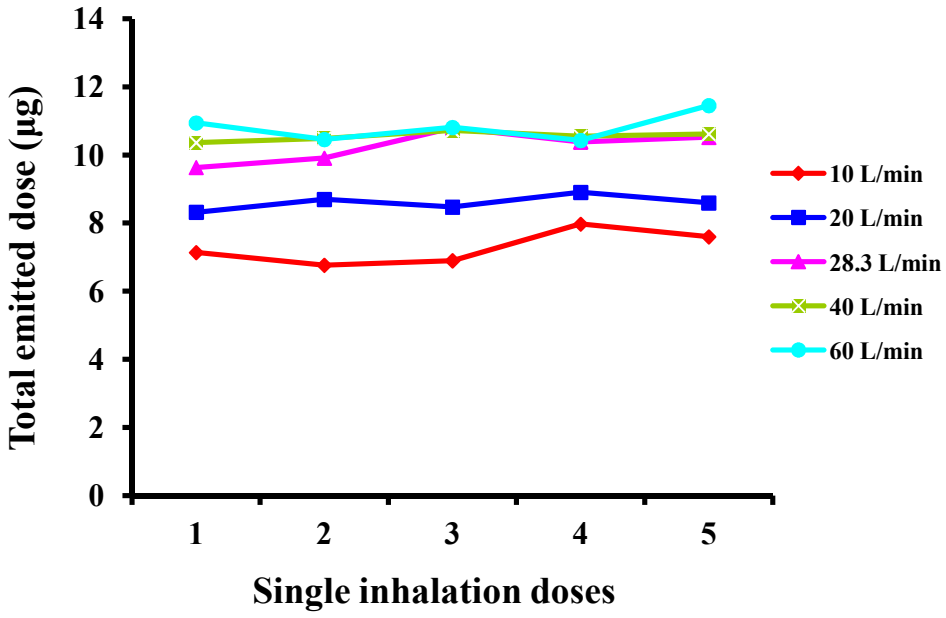

Figure 4.19: Mean emitted dose (µg) from one and two inhalations of formoterol fumarate from Easyhaler at varying inhalation flow rates (10-60 L/min) using inhalation volume of 4 L.

Table 4.6: Dose emitted (μg) Easyhaler® at the different inhalation flow rates of 10, 20, 28.3, 40 and 60 L/min using inhalation volume of 2 L.

Inhalation rates	one Inhalation	two Inhalations
10 L/min		
1	4.56	4.69
2	4.39	5.00
3	4.48	4.89
4	5.04	5.04
5	4.81	4.61
Mean	4.66	4.84
S.D.	0.26	0.19
%	38.80	40.37
20 L/min		
1	5.62	6.01
2	5.88	6.40
3	5.72	5.55
4	6.00	5.83
5	5.80	5.72
Mean	5.80	5.90
S.D.	0.14	0.33
%	48.36	49.18
28.3 L/min		
1	6.42	6.75
2	6.60	7.27
3	7.17	6.77
4	6.90	6.90
5	6.99	7.38
Mean	6.82	7.01
S.D.	0.31	0.29
%	56.81	58.45

40 L/min

1	6.91	7.18
2	7.01	7.15
3	7.17	7.37
4	7.05	7.22
5	7.13	7.31
Mean	7.05	7.25
S.D.	0.10	0.09
%	58.79	60.38

60 L/min

1	7.32	7.69
2	7.04	7.58
3	7.26	7.87
4	7.05	7.50
5	7.70	7.34
Mean	7.27	7.60
S.D.	0.27	0.20
%	60.60	63.31

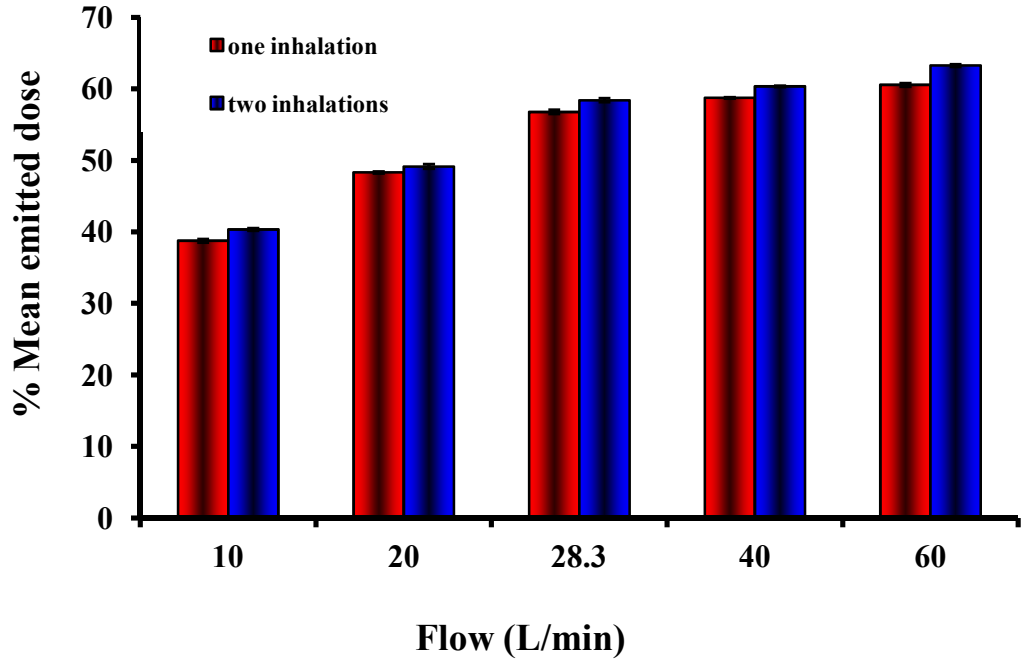

Figure 4.20: Mean emitted dose (%) from one and two inhalations of formoterol fumarate from Easyhaler® at varying inhalation flow rates (10-60 L/min) using inhalation volume of 2 L.

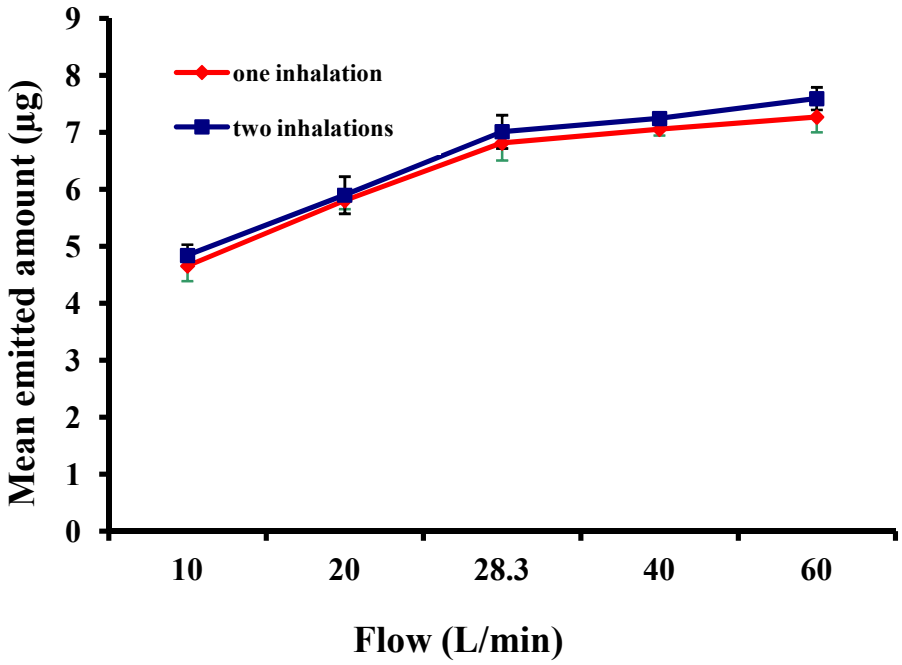

Figure 4.21: Mean emitted dose (µg) from one and two inhalations of formoterol fumarate from an Easyhaler® at varying inhalation flow rates (10-60 L/min) using inhalation volume of 2 L.

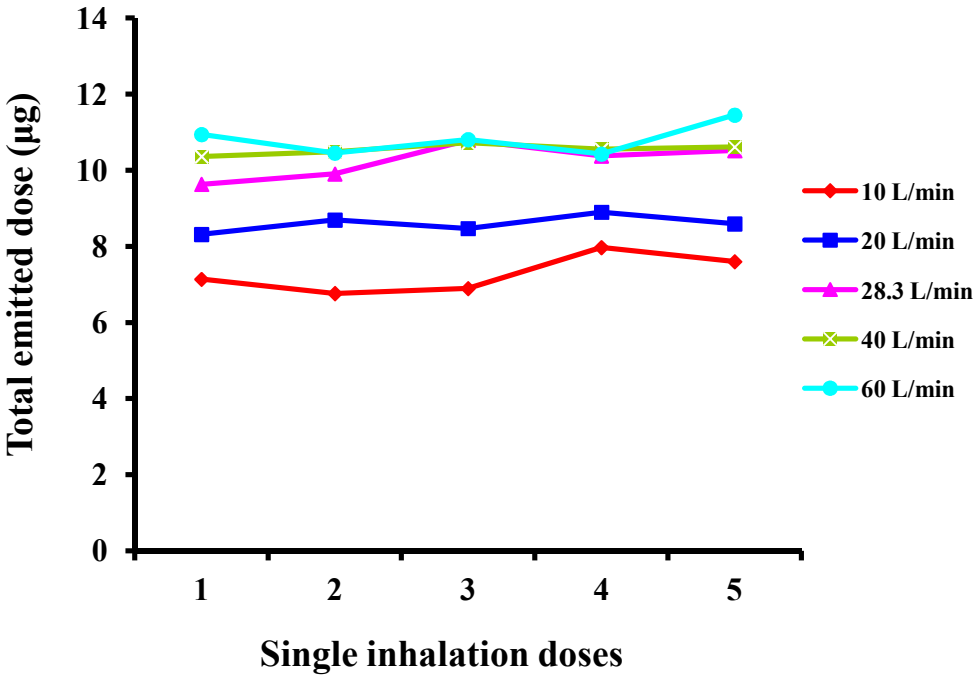

Figure 4.22: Total dose emitted (µg) of 5 separate single inhalation doses of formoterol fumarate from an Easyhaler® at different inhalation flow rates of 10, 20, 28.3, 40, and 60 L/min and inhalation volumes of 2 L.

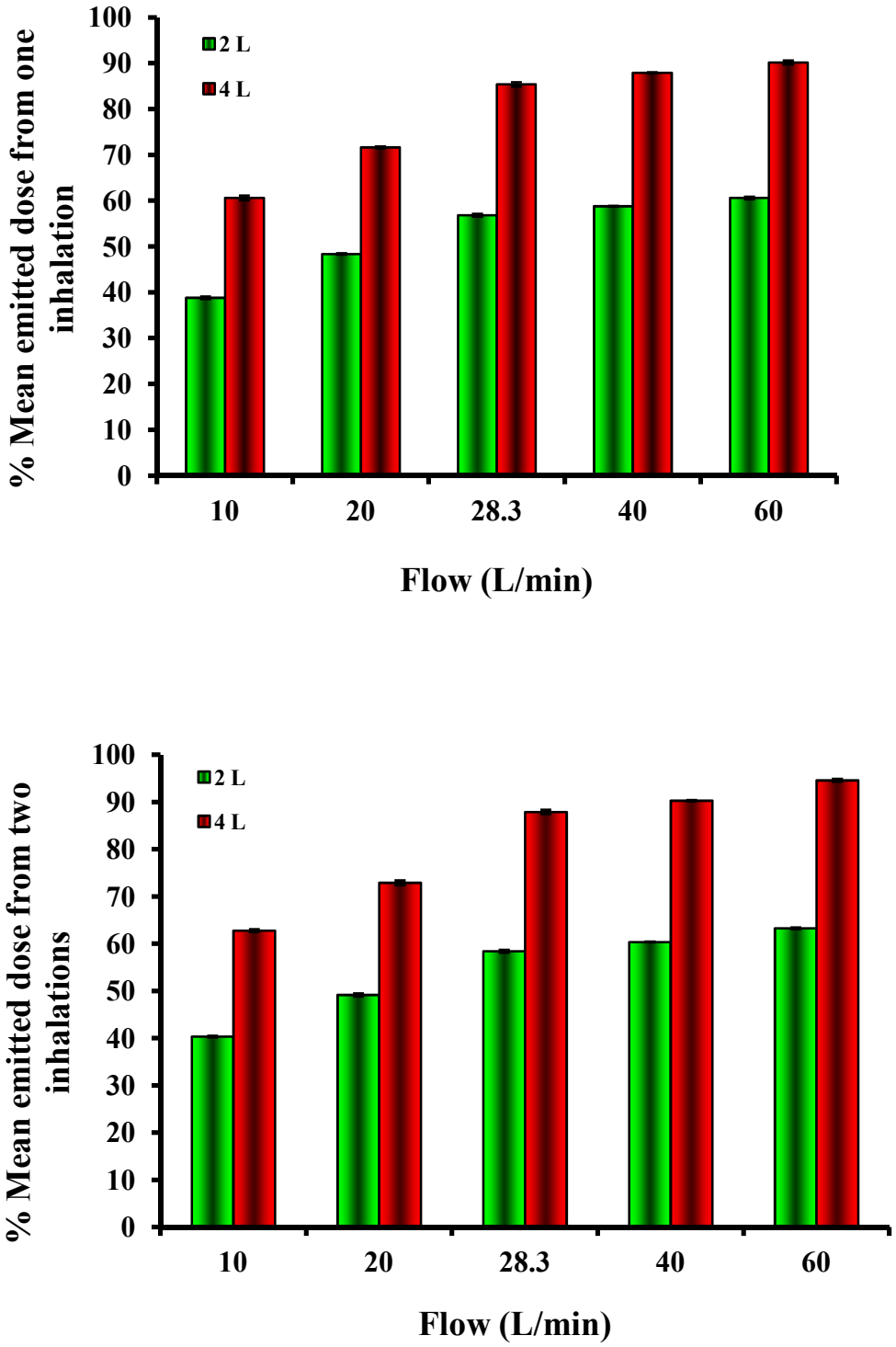

Figure 4.23: Comparison of mean emitted dose (%) from one and two inhalations of formoterol fumarate from an Easyhaler® at varying inhalation flow rates (10-60 L/min) using inhalation volumes of 4 L and 2 L.

Table 4.7: Statistical comparison of the % mean nominal dose emitted from the Aeroliser® at varying flow rates of 10, 15, 20, 28.3, 60, 90 L/min using inhalation volumes of 4 L and 2 L.

Inhalation flow rates (L/min)	**4 L** **% Mean nominal dose**	**2 L** **% Mean nominal dose**
10 vs 15 L/min	***p<0.001	*p<0.05
10 vs 20 L/min	***p<0.001	*p<0.05
10 vs 28.3 L/min	***p<0.001	***p<0.001
10 vs 60 L/min	***p<0.001	***p<0.001
10 vs 90 L/min	***p<0.001	***p<0.001
15 vs 20 L//min	***p<0.001	***p<0.001
15 vs 28.3 L/min	***p<0.001	***p<0.001
15 vs 60 L/min	***p<0.001	***p<0.001
15 vs 90 L/min	***p<0.001	***p<0.001
20 vs 28.3 L/min	**p<0.01	**p<0.01
20 vs 60 L/min	***p<0.001	***p<0.001
20 vs 90 L/min	***p<0.001	***p<0.001
28.3 vs 60 L/min	**p<0.01	*p<0.05
28.3 vs 90 L/min	***p<0.001	**p<0.01
60 vs 90 L/min	**p<0.01	p=0.243

Table 4.8: Statistical comparison of the % mean nominal dose emitted from the Aeroliser® using inhalation volumes of 2 L and 4 L at flows of 10, 15, 20, 28.3, 60, 90 L/min.

	% Mean nominal dose
4 L vs 2 L 10 L/min	***p<0.001
4 L vs 2 L 15 L/min	***p<0.001
4 L vs 2 L 20 L/min	***p<0.001
4 L vs 2 L 28.3 L/min	***p<0.001
4 L vs 2 L 60 L/min	***p<0.001
4 L vs 2 L 90 L/min	***p<0.001

Table 4.9: Statistical comparison of % mean nominal dose emitted from Aeroliser® after one and two inhalations.

	4 L **% Mean nominal dose**	**2 L** **% Mean nominal dose**
One vs two inhalations 10 L/min	***p<0.001	**p<0.01
One vs two inhalations 15 L/min	***p<0.001	**p<0.01
One vs two inhalations 20 L/min	***p<0.001	***p<0.001
One vs two inhalations 28.3in	***p<0.001	***p<0.001
One vs two inhalations 60 L/min	***p<0.001	***p<0.001
One vs two inhalations 90 L/min	**p<0.01	*p<0.05

Table 4.10: Statistical comparison of the % mean nominal dose emitted from the Oxis Turbuhaler® at varying flow rates of 10, 20, 28.3, 40, 60 L/min using inhalation volumes of 4 L and 2 L.

Inhalation flow rates (L/min)	**4 L % Mean nominal dose**	**2 L % Mean nominal dose**
10 vs 20 L/min	***p<0.001	***p<0.001
10 vs 28.3 L/min	***p<0.001	***p<0.001
10 vs 40 L/min	***p<0.001	***p<0.001
10 vs 60 L/min	***p<0.001	***p<0.001
20 vs 28.3 L//min	*p<0.05	***p<0.001
20 vs 40 L/min	***p<0.001	***p<0.001
20 vs 60 L/min	***p<0.01	***p<0.001
28.3 vs 40 L/min	***p<0.001	***p<0.001
28.3 vs 60 L/min	**p<0.01	***p<0.001
40 vs 60 L/min	p=0.729	p=0.729

Table 4.11: Statistical comparison of the % mean nominal dose emitted from the Oxis Turbuhaler® using inhalation volumes of 2 L and 4 L at flows of 10, 20, 28.3, 40, 60 L/min.

	% Mean nominal dose
4 L vs 2 L 10 L/min	***p<0.001
4 L vs 2 L 20 L/min	***p<0.001
4 L vs 2 L 28.3 L/min	***p<0.001
4 L vs 2 L 40 L/min	***p<0.001
4 L vs 2 L 60 L/min	***p<0.001

Table 4.12: Statistical comparison of % mean nominal dose emitted from Oxis Turbuhaler® after one and two inhalations.

	4 L % Mean nominal dose	**2 L % Mean nominal dose**
One vs two inhalations 10 L/min	p=0.510	*p<0.05
One vs two inhalations 20 L/min	p=0.985	*p<0.05
One vs two inhalations 28.3 L/min	p=0.098	*p<0.05
One vs two inhalations 40 L/min	p=0.240	*p<0.05
One vs two inhalations 60 L/min	p=0.191	**p<0.01

Table 4.13: Statistical comparison of the % mean nominal dose emitted from the Easyhaler® at varying flow rates of 10, 20, 28.3, 40, 60 L/min using inhalation volumes of 4 L and 2 L.

Inhalation flow rates (L/min)	**4 L** **% Mean nominal dose**	**2 L** **% Mean nominal dose**
10 vs 20 L/min	***p<0.001	*p<0.05
10 vs 28.3 L/min	***p<0.001	*p<0.05
10 vs 40 L/min	***p<0.001	***p<0.001
10 vs 60 L/min	***p<0.001	***p<0.001
20 vs 28.3 L//min	***p<0.001	***p<0.001
20 vs 40 L/min	***p<0.001	***p<0.001
20 vs 60 L/min	***p<0.001	***p<0.001
28.3 vs 40 L/min	**p<0.001	**p<0.01
28.3 vs 60 L/min	***p<0.001	***p<0.001
40 vs 60 L/min	p=729	*p<0.05

Table 4.14: Statistical comparison of the % mean nominal dose emitted from the Easyhaler® using inhalation volumes of 2 L and 4 L at flows of 10, 20, 28.3, 40, 60 L/min.

	% Mean nominal dose
4 L vs 2 L 10 L/min	***p<0.001
4 L vs 2 L 20 L/min	***p<0.001
4 L vs 2 L 28.3 L/min	***p<0.001
4 L vs 2 L 60 L/min	***p<0.001
4 L vs 2 L 90 L/min	***p<0.001

Table 4.15: Statistical comparison of % mean nominal dose emitted from Easyhaler® after one and two inhalations.

	4 L **% Mean nominal dose**	**2 L** **% Mean nominal dose**
One vs two inhalations 10 L/min	p=0.29	p=0.104
One vs two inhalations 20 L/min	p=0.36	p=0.361
One vs two inhalations 28.3 L/min	p=0.25	p=0.259
One vs two inhalations 40 L/min	*p<0.05	*p<0.05
One vs two inhalations 60 L/min	*p<0.05	*p<0.05

Table 4.16: Comparison of % mean nominal dose emitted from Foradil Aeroliser®, Oxis Turbuhaler®, ad Easyhaler®.

DPI	10 L/min	20 L/min	28.3 L/min	40 L/min	60 L/min	90 L/min
Aeroliser®	44.04 (0.28) %	59.42 (0.53) %,	73.13 (0.81) %	-	80.97 (0.42) %	88.09 (0.57) %
Turbuhaler®	44.04 (0.05) %	57.44 (0.88) %,	63.65 (1.57	67.07 (1.34) %	73.16 (2.62) %	-
Easyhaler®	60.63 (0.5) %	71.63 (1.84) %	85.38 (3.91) %,	87.89 (1.11) %	90.15 (3.49) %	-

4.4 DISCUSSION

Due to the variety of parameters involved in determining deposition of DPI within the lungs including output of the device per inhalation, the efficacy of de-aggregation of the drug, and the influence of lung disease, the efficacy of such devices should be analysed in in vivo studies in human volunteers under realistic conditions. Nonetheless, due to the time and cost involved in such techniques, in vitro techniques was introduced as an alternative approach for device characterisation that helps to estimate lung deposition (Meyer et al, 2004). The in vitro determination of the dose emitted at different inhalation flows and inhaled volumes provide an estimation of the doses emitted during normal practice. Weuthen et al (2002) have compared the in vitro aerosol performance and dose uniformity of Foradil Aeroliser® and Oxis Turbuhaler®. The study reported a mean total dose emitted at inhalation flow rates of 28.3, 40, 60, and 80 L/min over a steady inhalation volume of 4 L through the Oxis Turbuhaler® and Foradil Aeroliser®.

The (SD) mean emitted dose for Foradil Aeroliser were 71.41 (6.27) %, 69.54 (0.54) %, 81.73 (0.87) %, and 78.13 (7.52) %, respectively. The dose emission for Oxis Turbuhaler® were 58.44 (3.23 %, 60.74 (12.82) % 68.49 (8.66) %, and 57.88 (3.74) %, respectively. In the present study similar results were achieved for Foradil Aeroliser® with the (SD) mean % emitted dose of 59.42 (0.53) %, 73.13 (0.81) %, 80.97 (0.42) %, 88.09 (0.57) % at 20, 28.3, 60, and 90 L/min, respectively and 57.44 (0.88) %, 63.65 (1.57) %, 67.07 (1.34) %, and 73.16 (2.62) % at 20, 28.3, 40, and 60 L/min, respectively for Turbuhaler®. Additionally, results achieved for Easyhaler® with the (SD) mean % emitted dose were 71.63 (1.84) %, 85.38 (3.91) %, 87.89 (1.11) %, 90.15 (3.49) % at 20, 28.3, 40, and 60 L/min, respectively (Table 4.16 and Figures 4.2, 4.10, and 4.17 respectively).

Such results demonstrate that greater formoterol dose emission is achieved through both Easyhaler® and Foradil Aeroliser® devices compared to Turbuhaler®. Studies by Weuthen et al (2002) comparing the performance of DPI devices demonstrated that Turbuhaler® delivers about 16% less formoterol aerosol than Aeroliser®. Foradil Aeroliser® delivered more constant doses of approximately 80% of the nominal dose at flow rates of 60 L/min or higher. This correlates with the present work where the emitted dose of formoterol from a Foradil Aeroliser® was approximately 81% of the nominal dose at flow rates of 60 L/min (Figure 4.2). The different inhalation flow rates have also been shown to have an effect on dose delivery. The data on all three DPI devices (Foradil Aeroliser®, Turbuhaler®, and Easyhaler®) performance after first inhalation show a tendency for lower flows to produce reduced dosing and lower deposition of drug. Furthermore, as the flow rate increased, higher dose emission was obtained. Since, the energy created inside a DPI to generate the respirable dose is a product of inhalation flow and the inhaler's internal resistance, hence the faster the flow rate the higher will be the energy. Therefore, as DPI has flow dependent dose emission,

then the faster the inhalation rate the greater the drug de-aggregation and the better the quality of the emitted dose.

In line with this, Chan and Chew (2004) have measured emitted dose uniformity using a sampling apparatus described in the British Pharmacopaeia. Ten individual doses were collected from the Aeroliser® and the Turbuhaler®. Results showed that the Aeroliser® showed a slight dependence of the emitted dose on the air flow, with the average emitted dose increased from 80% (at 30 L/min) to 90% (at higher flows) of the 12-μg label claim as compared with 60% for the Turbuhaler®.

Other in vitro studies on the dose emission performance of pulmicort Turbuhaler® containing budesonide were performed at varying air inhalation flows. Results showed an increase in the fine particle mass when the flow is increased from 30 to 60 L/min (Ross and Schultz, 1996). A similar study by Malton et al (1996) has also shown the increased flow dependency property of Turbuhaler® containing terbuline.

In clinical terms, however, although patients are instructed to inhale as fast as they can and for as long as they can, for some patients inhaling too fast may result in high oropharyngeal impaction and low lung deposition (Chrystyn et al, 2009). This is particularly important for inhalers with low resistance such as Foradil Aeroliser®, where the inspiratory effort required generating high flows are relatively lower compared to that of a high resistance device. Nonetheless, the particle loss in airways by impaction at higher air flows is expected to be less for the better dispersed drug particles.

In study by Chew and Chan et al (2001) comparing performance of Aeroliser® and Turbuhaler® in performing formoterol aerosols results showed that at higher flows of 90 and 120 L/min for both inhalers generated very similar amounts of fine particles, which was about 30% of the label claim for the total dose. At lower flows of 30 and 60

L/min both inhalers generated significantly less fine particle in the aerosols, with Oxis Turbuhaler® producing significantly lesser amounts than the Foradil Aeroliser® at 30 L/min. These data is in agreement with results in the present study where formoterol dose emission of a Foradil Aeroliser® was greater than that of a Turbuhaler® at lower inhalation flows (Table 4.16 and Figures 4.2, 4.10). This is indeed expected since both these inhalers have different resistance to air flow. Foradil Aeroliser® device has low internal resistance whereas Oxis Turbuhaler® has a relatively high internal resistance. Thus, for a given inspiratory effort the air through the Foradil Aeroliser® will generate higher dose emission compared to Turbuhaler® (Chew and Chan, 2001). Easyhaler® has a high internal resistance, however the design of this new multidose DPI has focused on minimising the flow-dependent dose emission that occurs with the Turbuhaler® (Chrystyn, 2007). Studies by Malmberg et al (2010) were aimed at evaluating the ability of COPD patients to generate sufficient inspiratory flows through Easyhaler®. Results have shown that almost all COPD patients (98%), with various degree of airway obstruction were able to generate sufficient inspiratory flow through Easyhaler® at inhalation flow rates of 28.3 L/min. Furthermore, the study concluded that despite the high resistance of the inhaler, the great majority of COPD patients were able to achieve sufficient inspiratory flows through Easyhaler for optimal drug delivery to the lower airways. In the present study the formoterol dose emission from Easyhaler® was approximately 85.38 (3.91) % of the nominal dose at inhalation flow rates of 28.3 L/min, which is relatively higher than that achieved by Foradil Easyhaler® 73.13 (0.81) %, and much greater that the amount achieved through a Turbuhaler® %, 63.65 (1.57) % at the same flow rate (Tables 4.16 and Figures 4.17, 4.2, and 4.10).

The present study also compared the total emitted dose using different inhalation volumes of 2 L and 4 L. For the determination of the total dose emission the Pharmacopeias (USP, 2007, EP, 2005) recommend an inhalation of 4 L through the

inhaler with the inhalation flow maintained at constant rate that corresponds to the pressure drop of 4 kPa across the inhaler. However, conditions such as obstructive disease tend to decrease inhalation volume to critical values of around 2 L. Data produced from this study demonstrated that the emitted dose obtained from 4 L inhalation volume was significantly ($p<0.001$) higher than the amount obtained using 2 L for Foradil Aeroliser®, Oxis Turbuhaler®, and Easyhaler® (Figures 4.8, 4.16, and 4.23).

It is important therefore to be aware of the inhalation flows and the nature of inhalation profiles that different groups of patients can generate through different devices. In the present study, the determination of dose emission was extended to a second inhalation per each single dose. For DPI devices such as Foradil Aeroliser®, the drug is formulated as a single dose into a capsule inside the device and the dose emission from the reservoir occurs immediately at the start of the inhalation. Hence not only it is important to determine the dose emission at the start of the inhalation but also the inhalation volume that is required to empty the capsule. As a result it is common for the manufacturers to instruct patients to make two separate inhalations for each dose. In line with this, results have shown that after two inhalations there was an increase in formoterol dose emission from Foradil Aeroliser® at lower flows, however, higher flow rates of 60 and 90 L/min showed reduced dose emission after two inhalations (Table 4.1).

Oxis Turbuhaler® showed that following two inhalations an increase in formoterol dose emission was only observed for 2 L inhalation volume and not for 4 L. Such results suggest that a inhaling twice through a Turbuhaler® device may be suitable to those suffering from obstructive disease as their inhalation volume tend to decrease to critical values (Figures 4.10 and 4.11). Easyhaler® showed that following two inhalations an increase in dose emission was only observed at higher inhalation rates of 40 and 60

L/min but not at lower inhalation flows of 10 and 20 L/min (Figures 4.17 and 4.18). Furthermore, the present study has also focused on determination of inhaler dose uniformity. The delivered dose of DPI may considerably vary among different batches. According to the European Pharmacopeia uniformity of delivered doses complies with the requirements if 9 out of 10 results are within average of 75 to 125 % of the mean delivered dose. In the present study, the 10 single doses were emitted for Foradil and 5 doses for Oxis Turbuhaler® and Easyhaler® using a defined flow rate (Figures 4.4, 4.7, 4.12, 4.15, 4.19, and 4.22). There were no significant differences reported on the uniformity of the doses emitted. This is indeed desirable as uniformity of dose emission is one of the main factors influencing dosing efficiency of a DPI.

4.5 Conclusion

In the present study in-vitro dose emission of Foradil Aeroliser®, Oxis Turbuhaler®, and Easyhaler® delivery systems has demonstrated stable and reproducible drug delivery characteristics. Equally data from the Foradil Aeroliser® inhaler performance has demonstrated the need to inhale twice from each single dose to maximise dose emission and ensure effective emptying of the capsule content. Turbuhaler® performance showed the need to inhale twice when the inhalation volumes are critical (2 L). Interestingly, Easyhaler® inhaler performance showed the need to inhale twice only at higher flow inhalation rates. Furthermore, for all three DPI devices (Foradil Aeroliser®, Oxis Turbuhaler®, and Easyhaler®) the mean dose emission was shown to be dependent on inspiratory flow rate with higher flow rates producing greater dose emission. Additionally, a more effective dose emission was achieved at inhalation volumes of 4 L compared with inhalation volume of 2 L in all three devices.

CHAPTER FIVE

IN-VITRO AERODYNAMIC PARTICLE SIZE DISTRIBUTION OF OXIS TURBUHALER®, FORADIL AEROISER® AND EASYHALER® AT DIFFERENT INHALATION FLOW RATES AND INHALATION VOLUMES USING MIXIXG INLET

5.1 Introduction

Dry powder inhalers (DPIs) are an established technology for the delivery of drugs as aerosols to the lungs (Crompton 1991). The primary advantage of DPIs is their breath-actuated system hence only releasing drugs when the patient inhales. As a result the major disadvantage of the passive DPI is that energy delivered from patient inspiration is the only energy available for aerosol generation. Therefore, drug delivered from DPI to the lungs is primarily affected by the patient's ability to inhale appropriately (Newman et al 1988 Hickey et al 1994). Currently, inhaled formoterol DPI products are marketed as Foradil Aeroliser® (Norvatis Pharma), Turbuhaler® (Astra Zeneca), and Easyhale®r (Orion). DPI devices have varying internal resistance, require sufficient inspiratory flows, and consequently are dependent on the inspiratory effort of the patient (Clark and Hollingworth, 1993). In line with this, the United States Pharmacopoeia (USP) and the European Pharmacopeia (EP) have established guidelines for measuring of aerodynamic particle size of drugs delivered from DPI devices. Cascade impactors are one of the main tools used to determine the in vitro aerodynamic parameters of the dose emitted from inhaler devices. These in vitro parameters provide an insight into the potential for particle deposition in the lungs. The Anderson Cascade Impactor is described in the USP 2007 and EP 2005, and it is usually the method of choice to characterise the quality of the dose emitted from an inhaler.

The Anderson Cascade impactor has been designed to operate at inhalation flows of 28.3 L/min. Use of different inhalation flows alters the cut-off diameter of each stage of the Anderson Cascade Impactors. Recently, modifications to the stages of the Anderson Cascade Impactor have been introduced. This includes replacing some of the stages (Mitchell and Nagel, 2003). For instant, Anderson Cascade Impactor operating at 28.3 L/min would have nine separate stages from 0, 1, 2, 3, 4, 5, 6, 7, 8 and cut-off diameters of 9,5.8, 4.7, 3.3, 2.1, 1.1, 0.7, 0.4 μm (filter), respectively. However, when operating at

60 L/min the stages would be replaced to -1, 0, 1, 2, 3, 4, 5, 8 corresponding to cut-off diameters of 8.6, 6.5, 4.4, 3.2, 1.9, 1.2, 0.55, 0.26 μm (filter) (EP, 2005, USP, 2007). In line with this, despite the introduction of the stage replacements unless the cut-off diameters are re-calculated, the dose emission is restricted to 28.3, 60, and 90 L/min. In some occasions, if an inhaler has high resistance then patients with reduced inspiratory capacity may not be able to generate an inhalation flow of 28.3 L/min. It is therefore important to determine the dose emission characteristics below flow rates of 28.3 L/min. In order to improve the limitations of the Anderson Cascade Impactor and obtain values between the standard rates, a mixing inlet valve was introduced (Nadarassan, 2010). The mixing inlet valve with the Anderson Cascade Impactor (ACI) was set to operate at a flow of 60 L/min. This means that it is possible to identify the dose emission from an inhaler at a rate up to 60 L/min. The mixing inlet valve allows supplementary air flow from side channel to make up the difference between the air pulled through the inhaler and the Cascade Impactor. Additionally, the Anderson cascade Impactor is always kept at the same rate hence eliminating the need for calculations required to standardise the cut-off diameter of each stage.

Comparative studies by Weuthen et al (2002) were carried out between Foradil Aeroliser® and Oxis Turbuhaler® inhalers using an eight stage Anderson Cascade Impactor to determine the fine particle dose and mass median aerodynamic diameters (MMAD) at varying flows (28.3, 40, 60, and 90 L/min). However, the effect of inhalation flow rates below 28.3 L/min on fine particle deposition was not carried out whereas it has been previously reported that many patients with COPD are not able to achieve an inhalation flow of 28.3 L/min though the Turbuhaler (Tarsin, 2005). Moreover, recent studies on the effects of one and two inhalations from each dose have been suggested to significantly increase dose emission from a single dose (Abdelrahim et al, 2009). This is indeed desirable, especially for patients whose inspiratory flow

while inhaling tends to be relatively low. However, there have been no reports of in vitro studies comparing fine particle dose emission after one and two inhalations for formoterol products.

Therefore, the present study was designed to identify and compare lung deposition and particle size distribution of formoterol from three DPI devices (Oxis Turbuhaler®, Foradil Aeroliser®, Easyhaler®) at varying flow rate of 10, 20, 28.3, 40, 60, and 90 L/min using inhalation volumes of 4 L and 2 L. This was performed using Anderson Cascade Impactor with mixing inlet valve. The study also focused on determining the total drug delivery after one and two inhalations from each dose for all three DPI devices.

5.2 Methods and Instrumentation

5.2.1 Equipment and inhalation device

The various equipment used for the Anderson Cascade Impactor study are explained in Chapter 3 (section 3.14 and 3.15).

5.2.2 Instrumentation

5.2.2.1 Procedure to set up Anderson Cascade Impactor

The parts of the Anderson Cascade Impactor and its accessories (pre-separator and induction port) were washed with methanol and dried at room temperature. The collection plates were then sprayed with silicone and then allowed to dry for 1 hour prior to analysis. After the drying process the Anderson Cascade Impactor stages were assembled with 10 mL of 20 % methanol placed in the pre-separator and a GF 50

(Copley Scientific Ltd, UK) filter located in the final stage. The USP (2007) recommends the use of the pre-separator for DPI to leads large particles usually >10 µm (Mitchell, 2003). This would include those particles of the carrier, drug still attached to its carrier or large drug particle. A schematic design is shown in Figure 5.1. The inhalation time was set as explained in section 4.1 (Chapter 4).

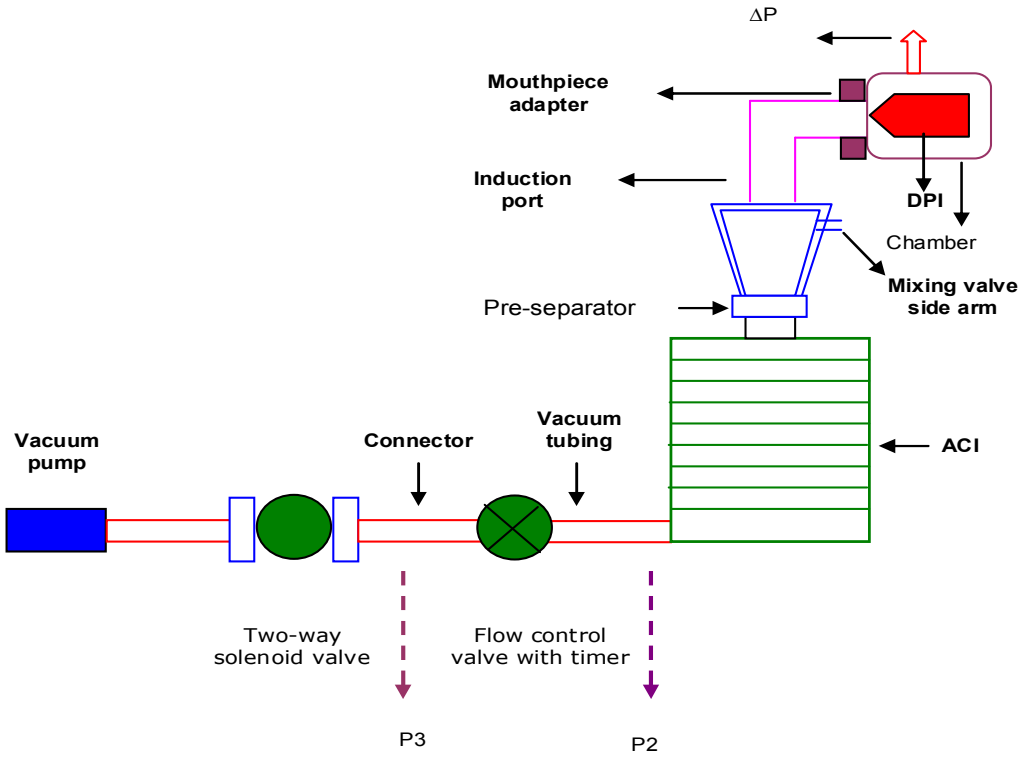

Figure 5.1: Anderson Cascade Impactor set-up for particle size analysis.

Dose emission from the Oxis Turbuhale®r, Foradil Aeroliser®, and Easyhaler® containing 12 µg formoterol were determined. Each individual device was attached to the induction port of the Anderson Cascade using the chamber to measure the pressure drop (ΔP). The flow control valve was adjusted until the flow, measured by the calibrated flow meter (MLS) attached to the chamber achieved a steady flow through

the system. Air volume of 4L was drawn through the inhaler for each determination and sonic flow (absolute pressure ratio P3/P2 <0.5) was confirmed as described in section 4.2.3 (Chapter 4). The EP 2005 and USP 2007 recommend the measurement of the emitted dose characteristics at a constant flow volume of 4 L through the inhaler, with a constant pressure drop of 4 KPa across the inhaler. In addition, inhalation volume of 2 L was also used. The determination from each inhaler was performed at a flow of 10, 20, 28.3, 40, 60, and 90 L/min accordingly. Ten consecutive doses were discharged into the Cascade Impactor from each inhaler device for measurement of dose emission characteristics. Additionally, ten doses were discharged into the Cascade Impactor consisting of first and second discharges from each dose emission to mimic first and second inhalation from a single dose. The dose was loaded as explained in the patient information leaflet.

The mixing inlet valve was connected between the induction port and pre-separator (Figure 5.2). The Anderson Cascade Impactor was set to be used at 60 L/min so stages 0 and 7 were replaced by -1 and -0. Thus, the cut-off diameter was not altered by using high flow rate. The mixing inlet valve side arm was closed by using parafilm. The inhaler devices were then attached to the induction port of the Anderson Cascade Impactor using the chamber. The Anderson Cascade Impactor apparatus was then connected to the critical flow controller (model TPK). The flow control valve was adjusted until the flow, measured by the flow meter (MKS) attached to the chamber was equal to 60 L/min (sonic flow was achieved P3/P2 ≤0.5). Then side arm of the mixing inlet valve was opened and the source of a compressed air was introduced through the side arm of the mixing inlet valve to compensate for the required flow of 60 L/min through the Anderson Cascade Impactor. For example to achieve a desired flow of 10 L/min through the inhaler device, a 50 L/min flow of compressed air was introduced through the side port of the mixing inlet valve (Figure 5.2).

A validation was performed in order to ascertain the flow through the inhaler and the mixing inlet valve. This was done by measuring the flow through the induction port using a mass flow meter and the corresponding pressure change (ΔP) was recorded (Nadarassan et al, 2010)

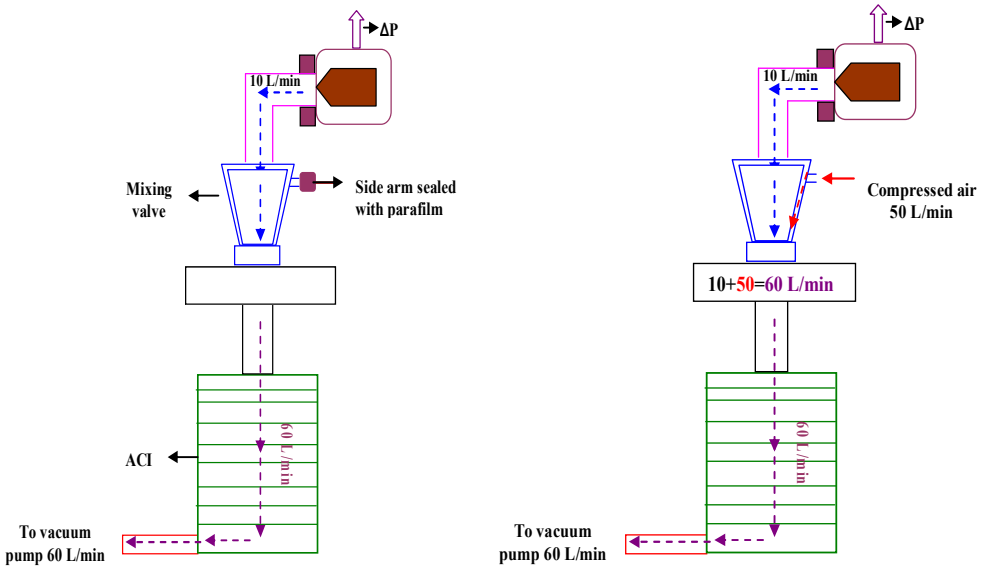

Figure 5.2: Inhalation flow set up using mixing inlet valve.

A total of three inhalers were used and the determination from each inhaler (Foradil Aeroliser®, Turbuhaler®, and Easyhaler®) was performed at flows of 10, 20, 28.3, 60 and 90 L/min for Foradil Aeroliser and at flows of 10, 20, 28.3, 40, and 60 L/min for Turbuhaler, and Easyhaler with a discharge time of 24, 12, 8.4, 6, 4, and 2.6 seconds, respectively that allow 4 L to be drawn through the inhaler. Inhalation volumes of 2 L and 4 L were used for all dose emissions. Ten consecutive doses were discharged for one inhalation analysis and for two inhalations analysis the ten doses were discharged once and twice from each dose emission.

5.2.3 High performance Liquid Chromatography (HPLC) analysis

The amount of the drug deposited in each stage of the Impactor was analysed using the HPLC as explained in Chapter 3 (section 3.39).

5.2.4 Fine particle analysis

The Copley Software (CITDAS version 2.0) was used to calculate the aerodynamic dose emission parameters. The stage cut sizes (μm) for the Anderson cascade Impactor at selected flows are described. The fine particle dose was calculated using the total mass of (R), of the drug deposited on stages and filter of the Cascade Impactor that captures the drug in the fine particle size range.

Fine particle dose = R/n

N=number of doses discharged Fine particle dose was the amount < 5μm Fine particle fraction is a ratio R (described above) with a total mass of drug delivered from the mouthpiece of the inhaler into the apparatus (∑A).

Fine particle fraction = R/∑A

The mass median aerodynamic diameter (MMAD) was obtained from the logarithm of the effective cut-off diameter corresponding to 50% undersize (on a log probability scale). The geometric standard deviation (GSD) was square root for the size corresponding to 15.87 % less than the stated size divided by square root of the size for 84.13%.

$GSD = \sqrt{d_{84.1}/d_{15.9}}$ (USP, 2007)

Where, d15.9 and d84.1 are the sizes corresponding to the mass-percentage values of 15.9% and 84.1%, respectively, for the cumulative size distribution.

5.2.3 Statistical analysis

Statistical analysis was carried out using one way Anova with the application of Bonferroni's correction to determine the differences between paired data.

5.3 Results

5.3.1 Comparison of aerodynamic fine particle dose between Aeroliser®, Turbuhaler®, Easyhaler®

In the present study, the effect of inhalation flow rate on particle size distribution and deposition on three DPI devices (Oxis Turbuhaler®, Foradil Aeroliser®, and Easyhaler®) were analysed. Moreover, inhalation volumes of 4 L and 2 L were compared at varying inhalation flow rates in all three devices. Lastly, the effects of one and two inhalations were also compared at different flow rates and inhalation volumes in all three DPI devices. The particle size distribution and fine particle dose (FPD), expressed as % nominal for formoterol at varying flows of 10, 20, 28.3, 60, and 90 L/min are shown in tables and figures. Abbreviations were used in table as follow:

TED = total emitted dose

FPD = fine particle dose < 5 μm

FPF = Fine particle fraction

MMAD = Mass median aerodynamic diameter

GSD = Geometric standard deviation

5.3.1.1 The effect of inhalation flow rate on particle size distribution and deposition in Foradil Aeroliser®.

Dose emission of fine particles from Foradil Aeroliser® was dependent on inhalation flow rate. The particle size distribution and fine particle dose (FPD), expressed as %

nominal for formoterol at varying flows of 10, 20, 28.3, 60 and 90 L/min were 9.23%, 14.70 %, 21.37%, 28.93%, and 39.70% at the inhalation volume of 4 L (one inhalation) and 4.17%, 5.55%, 7.28%, 8.41%, and 11.08% at the inhalation volume of 2 L (one inhalation), respectively (Figures 5.3-5.4 and Tables 5.1-5.20). The increase in inhalation flow rate showed significant ($p<0.01$-$p<0.001$) increase in the mean nominal fine particle dose at the varying inhalation flow rates using inhalation volumes of 4 L and 2 L (Figure 5.3 and Table 5.61). The performance of Foradil Aeroliser® in delivering formoterol showed a significant ($p<0.001$) decrease in MMAD with increase in inhalation flow rate (10, 20, 28.3 60, and 90 L/min) using inhalation volume of 4 L (6.22, 5.74, 4.44, 3.20, and 2.90 μm, respectively) and 2 L (6.52, 5.64, 4.96, 3.92, and 3.6 μm, respectively) (Figure 5.6 and Tables 5.1-5.20). There were also significant ($p<0.001$) differences in the fine particle dose achieved using a inhalation volume of 4 L as compared to 2 L at all inhalation flow rates. The amount of fine particle dose achieved was much greater at inhalation volumes of 4 L compared to 2 L (Figures 5.3-5.5 and Tables 5.1-5.20 and 5.62). Foradil Aeroliser® performance following one and two inhalations showed significant ($p<0.001$) increase in the mean nominal fine particle dose for two inhalations compared to one inhalation at inhalation volumes of 4 L and 2 L (Figures 5.3-5.4 and Tables 5.1-5.20).

Table 5.1: Aerodynamic particle profile from Foradil Aeroliser® following one inhalation at flows of 10 L/min and inhalation volume of 4 L.

Amount deposited on each stage (µg)						
Stage	**Run 1**	**Run 2**	**Run 3**	**Run 4**	**Run 5**	**Mean µg**
Induction port	11.87	10.63	11.12	10.48	10.72	10.96
-1	7.20	7.61	8.12	6.58	6.40	7.18
0	5.47	5.23	4.72	6.06	6.32	5.56
1	2.79	2.55	3.04	3.38	2.64	2.88
2	3.42	3.22	3.67	3.01	3.27	3.32
3	2.20	2.69	2.45	2.79	3.05	2.64
4	1.89	1.01	2.12	1.37	1.24	1.53
5	0.76	0.87	0.46	0.78	0.56	0.69
6	0.00	0.00	0.08	0.02	0.06	0.03
Filter	0.00	0.00	0.00	0.00	0.00	0.00
TED (µg)	53.23	50.96	52.91	51.01	51.59	51.94
TED µg/dose	5.32	5.10	5.29	5.10	5.16	5.19
TED %	44.36	42.47	44.09	42.51	42.99	43.28
%FPD (nominal)	9.22	8.62	9.85	9.46	9.02	9.23
%FPF (emitted)	20.78	20.29	22.34	22.25	20.97	21.33
MMAD (µm)	6.20	6.50	6.10	6.10	6.20	6.22
GSD	2.30	2.20	2.30	2.20	2.20	2.24

Table 5.2: Aerodynamic particle profile Foradil Aeroliser® following two inhalations at flows of 10 L/min and inhalation volume of 4 L.

Amount deposited on each stage (µg)						
Stage	**Run 1**	**Run 2**	**Run 3**	**Run 4**	**Run 5**	**Mean µg**
Induction port	16.22	16.11	16.76	15.86	15.96	16.18
-1	8.73	8.49	9.16	10.36	10.04	9.36
0	5.97	5.22	6.87	6.95	5.07	6.02
1	5.43	5.83	5.19	5.27	7.39	5.82
2	5.92	6.07	5.68	5.76	5.88	5.86
3	4.54	4.65	4.31	4.38	4.5	4.48
4	3.54	3.65	3.6	3.44	3.44	3.53
5	1.51	1.35	1.46	1.67	1.12	1.42
6	0.12	0.23	0.67	0.29	0.39	0.34
Filter	0.00	0.00	0.00	0.00	0.00	0.00
TED (µg)	80.57	80.25	83.81	82.98	82.30	81.98
TED µg/dose	8.06	8.03	8.38	8.30	8.23	8.20
TED %	67.14	66.88	69.84	69.15	68.58	68.32
%FPD (nominal)	17.55	18.15	17.43	17.34	18.93	17.88
%FPF (emitted)	26.14	27.14	24.95	25.08	27.61	26.18
MMAD (µm)	5.1	5	5.3	5.4	5.2	5.20
GSD	2.3	2.3	2.5	2.4	2.2	2.34

Table 5.3: Aerodynamic particle profile Foradil Aeroliser® following one inhalation at flows of 20 L/min and inhalation volume of 4 L.

Amount deposited on each stage (µg)						
Stage	**Run 1**	**Run 2**	**Run 3**	**Run 4**	**Run 5**	**Mean µg**
Induction port	12.74	11.85	12.50	11.60	11.70	12.08
-1	9.07	8.83	9.50	7.70	7.38	8.50
0	7.34	6.45	6.10	8.18	7.30	7.07
1	4.66	3.77	4.42	5.50	3.62	4.39
2	5.29	5.44	5.05	5.13	5.25	5.23
3	4.07	4.18	4.83	4.91	4.03	4.40
4	2.07	2.82	2.39	1.97	3.20	2.49
5	1.04	1.88	0.49	0.59	1.37	1.07
6	0.23	0.00	0.00	0.00	0.00	0.05
Filter	0.00	0.00	0.00	0.00	0.00	0.00
TED (µg)	64.88	62.81	63.17	64.36	61.14	63.27
TED µg/dose	6.49	6.28	6.32	6.44	6.11	6.33
TED %	54.07	52.34	52.64	53.63	50.95	52.73
%FPD (nominal)	14.47	15.08	14.32	15.08	14.56	14.70
%FPF (emitted)	26.76	28.80	27.20	28.12	28.57	27.89
MMAD (µm)	5.70	5.80	5.80	5.60	5.80	5.74
GSD	2.10	2.00	1.90	2.00	1.90	1.98

Table 5.4: Aerodynamic particle profile Foradil Aeroliser® following two inhalations at flows of 20 L/min and inhalation volume of 4 L.

Amount deposited on each stage (µg)						
Stage	**Run 1**	**Run 2**	**Run 3**	**Run 4**	**Run 5**	**Mean µg**
Induction port	11.97	11.08	12.73	12.83	12.93	12.31
-1	10.30	11.06	11.73	10.93	11.61	11.13
0	8.57	9.68	7.33	9.41	9.53	8.90
1	7.89	7.29	7.65	6.73	8.85	7.68
2	11.52	9.76	10.28	10.36	9.48	10.28
3	5.30	5.41	5.06	5.14	5.26	5.23
4	3.30	3.41	3.36	3.2	3.2	3.29
5	1.27	2.11	1.72	1.82	2.92	1.97
6	1.09	1.89	1.31	1.72	1.21	1.44
Filter	0.00	0.12	0.32	0.12	0	0.11
TED (µg)	90.04	87.77	89.84	91.50	91.74	90.18
TED µg/dose	9.00	8.78	8.98	9.15	9.17	9.02
TED %	75.03	73.14	74.87	76.25	76.45	75.15
%FPD (nominal)	25.31	24.99	24.75	24.24	25.77	25.01
%FPF (emitted)	33.73	34.13	33.06	32.14	33.70	33.35
MMAD (µm)	5.1	5.2	5	5.2	5.3	5.16
GSD	2.1	2.6	2.3	2.2	2.4	2.32

Table 5.5: Aerodynamic particle profile Foradil Aeroliser® following one inhalation at flows of 28.3 L/min and inhalation volume of 4 L.

Amount deposited on each stage (µg)						
Stage	**Run 1**	**Run 2**	**Run 3**	**Run 4**	**Run 5**	**Mean µg**
Induction port	13.97	14.08	14.39	12.65	14.44	13.91
-1	11.30	11.06	11.39	10.75	10.12	10.92
0	8.57	8.68	7.99	9.23	10.04	8.90
1	5.89	6.00	6.31	6.55	6.36	6.22
2	9.52	8.67	9.94	9.18	8.99	9.26
3	5.30	5.41	4.72	5.96	4.77	5.23
4	3.30	3.41	3.02	3.02	1.77	2.90
5	1.27	1.35	1.40	1.64	1.05	1.34
6	0.37	0.82	0.81	0.56	0.76	0.66
Filter	0.03	0.00	0.00	0.00	0.07	0.02
TED (µg)	81.35	81.53	81.64	83.42	81.14	81.82
TED µg/dose	8.14	8.15	8.16	8.34	8.11	8.18
TED %	67.79	67.94	68.03	69.52	67.62	68.18
%FPD (nominal)	21.40	21.38	21.83	22.43	19.81	21.37
%FPF (emitted)	31.57	31.47	32.09	32.26	29.30	31.34
MMAD (µm)	4.40	4.40	4.40	4.40	4.60	4.44
GSD	2.00	2.10	2.00	2.00	1.90	2.00

Table 5.6: Aerodynamic particle profile Foradil Aeroliser® following two inhalations at flows of 28.3 L/min and inhalation volume of 4 L.

Amount deposited on each stage (µg)						
Stage	**Run 1**	**Run 2**	**Run 3**	**Run 4**	**Run 5**	**Mean µg**
Induction port	17.20	17.2	16.1	17.12	17.18	16.96
-1	14.53	14.53	13.08	14.12	12.28	13.71
0	10.80	10.98	10.7	10.72	10.76	10.79
1	7.12	7.12	8.02	9.04	8.08	7.88
2	10.75	10.75	10.69	12.67	10.71	11.11
3	6.53	6.53	7.43	7.45	7.49	7.09
4	4.53	4.53	5.43	5.75	4.55	4.96
5	2.50	2.5	3.37	4.13	3.17	3.13
6	1.60	1.6	2.84	3.54	2.09	2.33
Filter	0.76	1.06	1.02	1.13	0.95	0.98
TED (µg)	109.61	110.51	110.98	115.30	108.90	111.06
TED µg/dose	10.96	11.05	11.10	11.53	10.89	11.11
TED %	91.34	92.09	92.48	96.08	90.75	92.55
%FPD (nominal)	28.16	28.41	32.33	36.43	30.87	31.24
%FPF (emitted)	30.83	30.85	34.96	37.91	34.01	33.71
MMAD (µm)	4.3	4.4	4	3.9	4.1	4.14
GSD	2.5	2.3	3.1	3.3	2.8	2.80

Table 5.7: Aerodynamic particle profile Foradil Aeroliser® following one inhalation at flows of 60 L/min and inhalation volume of 4 L.

Amount deposited on each stage (µg)						
Stage	**Run 1**	**Run 2**	**Run 3**	**Run 4**	**Run 5**	**Mean µg**
Induction port	15.34	15.45	15.76	14.02	15.81	15.28
-1	12.67	12.43	12.76	12.12	12.29	12.45
0	9.94	10.05	10.36	11.60	11.27	10.64
1	7.26	7.37	7.68	7.92	7.59	7.56
2	12.89	12.04	12.31	12.55	12.63	12.48
3	6.67	6.78	6.09	6.33	6.60	6.49
4	5.67	4.78	5.39	5.39	5.27	5.30
5	3.35	2.46	2.77	2.01	1.68	2.45
6	0.34	0.55	0.56	0.13	0.44	0.40
Filter	0.00	0.04	0.03	0.00	0.00	0.01
TED (µg)	99.70	97.74	99.12	98.69	98.75	98.80
TED µg/dose	9.97	9.77	9.91	9.87	9.88	9.88
TED %	83.08	81.45	82.60	82.24	82.29	82.33
%FPD (nominal)	30.15	28.35	29.03	28.61	28.51	28.93
%FPF (emitted)	36.29	34.81	35.14	34.79	34.64	35.13
MMAD (µm)	3.20	3.30	3.20	3.10	3.20	3.20
GSD	2.10	2.10	2.00	2.10	2.10	2.08

Table 5.8: Aerodynamic particle profile Foradil Aeroliser® following two inhalations at flows of 60 L/min and inhalation volume of 4 L.

Amount deposited on each stage (µg)						
Stage	**Run 1**	**Run 2**	**Run 3**	**Run 4**	**Run 5**	**Mean µg**
Induction port	16.34	16.68	17.43	15.29	16.92	16.53
-1	13.67	13.66	14.43	13.39	13.71	13.77
0	10.94	11.28	11.03	11.87	10.63	11.15
1	8.26	8.6	9.35	9.19	9.95	9.07
2	11.89	11.27	12.98	11.82	12.58	12.11
3	9.67	9.01	9.76	9.6	9.36	9.48
4	5.67	6.01	6.06	5.66	5.36	5.75
5	3.35	3.69	4.44	4.28	4.04	3.96
6	1.34	1.78	2.23	1.4	2.83	1.92
Filter	0.47	0.79	0.79	0.96	1.27	0.86
TED (µg)	109.17	110.02	116.25	113.06	114.60	112.62
TED µg/dose	10.92	11.00	11.63	11.31	11.46	11.26
TED %	90.98	91.68	96.88	94.22	95.50	93.85
%FPD (nominal)	33.88	34.29	38.01	35.76	37.83	35.95
%FPF (emitted)	37.24	37.40	39.23	37.95	39.61	38.29
MMAD (µm)	2.8	2.7	2.8	2.8	2.8	2.78
GSD	2.5	3	2.4	2.6	2.7	2.64

Table 5.9: Aerodynamic particle profile Foradil Aeroliser® following one inhalation at flows of 90 L/min and inhalation volume of 4 L.

Amount deposited on each stage (µg)						
Stage	**Run 1**	**Run 2**	**Run 3**	**Run 4**	**Run 5**	**Mean µg**
Induction port	16.12	17.09	16.24	17.30	16.80	16.71
-2	4.45	4.42	3.57	4.63	3.13	4.04
-1	7.72	7.69	7.84	7.90	8.40	7.91
0	8.38	8.35	8.50	8.56	8.06	8.37
1	10.01	10.98	10.13	10.19	10.69	10.40
2	15.44	15.41	15.56	15.62	15.12	15.43
3	7.79	7.76	7.91	7.97	7.47	7.78
4	3.47	3.44	3.59	3.65	3.15	3.46
5	1.46	1.43	1.58	2.64	1.14	1.65
Filter	0.56	0.53	0.68	0.74	0.24	0.55
TED (µg)	100.53	103.15	100.97	104.69	100.69	102.01
TED µg/dose	10.05	10.31	10.10	10.47	10.07	10.20
TED %	83.78	85.96	84.14	87.24	83.91	85.00
%FPD (nominal)	39.26	39.91	39.96	41.14	38.23	39.70
%FPF (emitted)	46.86	46.43	47.49	47.16	45.56	46.70
MMAD (µm)	2.90	2.90	2.90	2.90	2.90	2.90
GSD	1.90	1.90	1.90	2.10	1.90	1.94

Table 5.10: Aerodynamic particle profile Foradil Aeroliser® following two inhalations at flows of 90 L/min and inhalation volume of 4 L.

Amount deposited on each stage (µg)						
Stage	**Run 1**	**Run 2**	**Run 3**	**Run 4**	**Run 5**	**Mean µg**
Induction port	17.35	17.46	17.77	16.03	17.82	17.29
-2	6.68	6.44	6.77	6.13	6.5	6.50
-1	8.95	8.06	8.37	8.61	8.42	8.48
0	9.61	9.72	10.03	10.27	10.08	9.94
1	13.58	12.73	14	13.24	13.05	13.32
2	14.36	14.47	14.78	14.02	14.83	14.49
3	15.36	15.47	14.08	14.08	15.83	14.96
4	6.04	6.15	6.46	6.7	6.51	6.37
5	2.03	2.24	2.25	2.82	2.3	2.33
Filter	0.98	0.48	0.77	0.69	0.34	0.65
TED (µg)	122.53	121.03	123.71	122.23	125.21	122.94
TED µg/dose	12.25	12.10	12.37	12.22	12.52	12.29
TED %	102.11	100.86	103.09	101.86	104.34	102.45
%FPD (nominal)	51.63	51.05	51.98	51.52	52.45	51.73
%FPF (emitted)	50.57	50.62	50.42	50.58	50.27	50.49
MMAD (µm)	2.6	2.4	2.5	2.6	2.5	2.52
GSD	1.90	2.00	2.00	2.10	1.90	1.98

Table 5.11: Aerodynamic particle profile Foradil Aeroliser® following one inhalation at flows of 10 L/min and inhalation volume of 2 L.

Amount deposited on each stage (µg)						
Stage	**Run 1**	**Run 2**	**Run 3**	**Run 4**	**Run 5**	**Mean µg**
Induction port	6.17	6.94	6.83	8.31	7.56	7.16
-1	3.20	2.97	3.86	3.34	3.03	3.28
0	3.47	3.24	3.13	2.61	2.30	2.95
1	1.79	1.56	1.45	1.93	1.62	1.67
2	1.42	1.79	1.68	1.56	1.85	1.66
3	1.02	1.29	1.08	1.16	1.25	1.16
4	0.43	0.20	1.09	0.57	0.16	0.49
5	0.00	0.00	0.00	0.14	0.00	0.03
6	0.00	0.00	0.00	0.00	0.00	0.00
Filter	0.00	0.00	0.00	0.00	0.00	0.00
TED (µg)	24.98	26.01	27.92	28.38	27.91	27.04
TED µg/dose	2.50	2.60	2.79	2.84	2.79	2.70
TED %	20.82	21.68	23.27	23.65	23.26	22.53
%FPD (nominal)	3.88	4.03	4.42	4.47	4.07	4.17
%FPF (emitted)	18.65	18.61	18.98	18.89	17.48	18.52
MMAD (µm)	6.40	6.80	6.40	6.50	6.50	6.52
GSD	2.10	2.20	2.30	2.40	2.30	2.26

Table 5.12: Aerodynamic particle profile Foradil Aeroliser® following two inhalations at flows of 10 L/min and inhalation volume of 2 L.

Amount deposited on each stage (µg)						
Stage	**Run 1**	**Run 2**	**Run 3**	**Run 4**	**Run 5**	**Mean µg**
Induction port	11.87	11.41	12.85	12.99	12.13	12.25
-1	3.20	2.74	4.18	4.32	3.46	3.58
0	1.71	1.25	2.69	2.83	1.97	2.09
1	2.23	1.77	3.21	3.35	2.49	2.61
2	4.27	3.81	5.25	5.39	4.53	4.65
3	2.12	1.66	3.1	3.24	2.38	2.50
4	0.45	0.87	1.43	1.57	0.71	1.01
5	0.00	0	0	0	0	0.00
6	0.00	0	0	0	0	0.00
Filter	0.00	0.00	0.00	0.00	0.00	0.00
TED (µg)	45.48	42.22	54.30	55.56	47.82	49.08
TED µg/dose	4.55	4.22	5.43	5.56	4.78	4.91
TED %	37.90	35.18	45.25	46.30	39.85	40.90
%FPD (nominal)	7.56	6.76	10.83	11.29	8.43	8.97
%FPF (emitted)	19.94	19.21	23.92	24.39	21.14	21.72
MMAD (µm)	6.1	5.9	6.0	6.0	6.1	6.02
GSD	2.1	1.9	1.9	1.9	1.8	1.92

Table 5.13: Aerodynamic particle profile Foradil Aeroliser® following one inhalation at flows of 20 L/min and inhalation volume of 2 L.

Amount deposited on each stage (µg)						
Stage	**Run 1**	**Run 2**	**Run 3**	**Run 4**	**Run 5**	**Mean µg**
Induction port	8.74	7.97	7.10	6.62	6.33	7.35
-1	2.78	2.01	2.14	2.66	2.37	2.39
0	1.47	1.70	1.83	1.35	1.06	1.48
1	1.09	1.02	1.15	1.67	1.38	1.26
2	2.42	1.65	1.03	1.01	1.01	1.42
3	2.02	2.25	2.38	2.05	2.51	2.24
4	1.43	1.66	1.47	1.31	1.02	1.38
5	0.23	0.46	0.59	0.11	0.38	0.35
6	0.00	0.00	0.00	0.00	0.00	0.00
Filter	0.00	0.00	0.00	0.00	0.00	0.00
TED (µg)	31.55	30.55	29.78	25.91	24.61	28.48
TED µg/dose	3.16	3.06	2.98	2.59	2.46	2.85
TED %	26.29	25.46	24.82	21.59	20.51	23.73
%FPD (nominal)	5.99	5.87	5.52	5.13	5.25	5.55
%FPF (emitted)	22.79	23.04	22.23	23.74	25.60	23.48
MMAD (µm)	5.80	5.70	5.60	5.60	5.50	5.64
GSD	1.90	1.90	1.90	2.00	1.90	1.92

Table 5.14: Aerodynamic particle profile Foradil Aeroliser® following two inhalations at flows of 20 L/min and inhalation volume of 2 L.

Amount deposited on each stage (µg)						
Stage	**Run 1**	**Run 2**	**Run 3**	**Run 4**	**Run 5**	**Mean µg**
Induction port	13.97	13.81	12.30	11.64	12.74	12.89
-1	4.30	5.14	3.63	5.97	4.07	4.62
0	5.57	6.41	6.90	6.24	5.34	6.09
1	4.89	5.73	5.22	5.56	5.66	5.41
2	4.72	4.56	4.05	3.39	3.49	4.04
3	3.30	3.14	3.63	3.97	3.07	3.42
4	1.30	2.14	1.63	1.67	1.94	1.74
5	0.45	1.29	0.24	0.12	0.22	0.46
6	0.00	0.00	0.00	0.00	0.00	0.00
Filter	0.00	0.00	0.00	0.00	0.00	0.00
TED (µg)	60.70	66.10	63.46	60.10	58.27	61.73
TED µg/dose	6.07	6.61	6.35	6.01	5.83	6.17
TED %	50.58	55.08	52.88	50.08	48.56	51.44
%FPD (nominal)	12.22	14.05	12.31	12.26	11.98	12.56
%FPF (emitted)	24.15	25.51	23.27	24.48	24.68	24.42
MMAD (µm)	5.3	5.2	5.2	5.2	5.2	5.22
GSD	2.3	2.2	2.1	2.2	2.1	2.18

Table 5.15: Aerodynamic particle profile Foradil Aeroliser® following one inhalation at flows of 28.3 L/min and inhalation volume of 2 L.

Amount deposited on each stage (µg)						
Stage	**Run 1**	**Run 2**	**Run 3**	**Run 4**	**Run 5**	**Mean µg**
Induction port	8.63	8.50	8.38	9.09	8.75	8.67
-1	0.67	0.54	0.42	1.13	0.79	0.71
0	0.83	0.70	0.58	1.29	0.95	0.87
1	1.09	0.96	0.84	1.55	1.21	1.13
2	1.52	1.39	1.27	1.98	1.64	1.56
3	2.05	2.92	2.80	2.51	2.17	2.49
4	2.09	1.96	1.84	1.55	2.21	1.93
5	1.28	1.15	1.03	1.24	1.40	1.22
6	0.37	0.24	0.12	0.83	0.49	0.41
Filter	0.00	0.00	0.00	0.00	0.00	0.00
TED (µg)	31.36	29.93	29.61	31.92	31.68	30.90
TED µg/dose	3.14	2.99	2.96	3.19	3.17	3.09
TED %	26.13	24.94	24.68	26.60	26.40	25.75
%FPD (nominal)	7.00	7.18	6.58	8.05	7.60	7.28
%FPF (emitted)	26.79	28.80	26.68	30.26	28.79	28.26
MMAD (µm)	4.90	4.90	5.00	4.90	5.10	4.96
GSD	2.00	2.10	2.00	2.00	1.90	2.00

Table 5.16: Aerodynamic particle profile Foradil Aeroliser® following two inhalations at flows of 28.3 L/min and inhalation volume of 2 L.

Amount deposited on each stage (µg)						
Stage	**Run 1**	**Run 2**	**Run 3**	**Run 4**	**Run 5**	**Mean µg**
Induction port	15.20	15.48	16.07	14.83	15.74	15.46
-1	2.88	3.16	3.75	2.51	3.42	3.14
0	5.78	4.06	4.65	3.41	4.32	4.44
1	6.33	6.61	7.2	5.96	6.87	6.59
2	5.97	6.25	6.84	5.6	6.51	6.23
3	5.72	5.78	5.59	5.35	5.26	5.54
4	3.98	3.26	3.85	3.61	3.52	3.64
5	1.53	1.81	2.4	1.16	2.07	1.79
6	0.65	0.93	0.78	0.28	0.45	0.62
Filter	0.00	0.00	0.00	0.00	0.00	0.00
TED (µg)	74.33	74.19	79.16	68.26	75.53	74.29
TED µg/dose	7.43	7.42	7.92	6.83	7.55	7.43
TED %	61.94	61.83	65.97	56.88	62.94	61.91
%FPD (nominal)	20.15	20.53	22.22	18.30	20.57	20.35
%FPF (emitted)	32.53	33.21	33.68	32.17	32.68	32.85
MMAD (µm)	4.5	4.3	4.4	4.4	4.4	4.40
GSD	2.1	2	1.9	2.3	2.1	2.08

Table 5.17: Aerodynamic particle profile Foradil Aeroliser® following one inhalation at flows of 60 L/min and inhalation volume of 2 L.

Amount deposited on each stage (µg)						
Stage	**Run 1**	**Run 2**	**Run 3**	**Run 4**	**Run 5**	**Mean µg**
Induction port	9.56	9.44	10.02	9.34	9.25	9.52
-1	0.73	0.61	1.19	0.51	0.42	0.69
0	0.99	0.87	1.45	0.77	0.68	0.95
1	1.29	1.17	1.75	1.07	0.98	1.25
2	1.92	1.80	1.38	1.70	1.61	1.68
3	2.65	2.53	2.11	2.43	3.34	2.61
4	2.49	2.37	2.95	2.27	2.18	2.45
5	1.28	1.16	1.74	1.06	1.97	1.44
6	0.89	0.77	0.35	0.67	0.58	0.65
Filter	0.00	0.00	0.00	0.00	0.00	0.00
TED (µg)	32.83	31.51	34.89	30.41	31.42	32.21
TED µg/dose	3.28	3.15	3.49	3.04	3.14	3.22
TED %	27.36	26.26	29.08	25.34	26.18	26.84
%FPD (nominal)	8.77	8.17	8.57	7.67	8.88	8.41
%FPF (emitted)	32.04	31.10	29.46	30.25	33.93	31.36
MMAD (µm)	3.90	3.90	3.80	4.00	4.00	3.92
GSD	2.10	2.10	2.10	2.30	2.00	2.12

Table 5.18: Aerodynamic particle profile Foradil Aeroliser® following two inhalations at flows of 60 L/min and inhalation volume of 2 L.

Amount deposited on each stage (µg)						
Stage	**Run 1**	**Run 2**	**Run 3**	**Run 4**	**Run 5**	**Mean µg**
Induction port	16.01	16.35	16.279	16.73	15.95	16.26
-1	1.89	2.23	2.159	2.61	1.83	2.14
0	3.32	3.66	3.589	4.04	3.26	3.57
1	6.29	6.63	6.559	7.01	6.23	6.54
2	6.84	6.18	6.109	6.56	5.78	6.29
3	4.78	5.12	5.049	5.5	4.72	5.03
4	3.08	4.42	4.349	4.8	3.82	4.09
5	2.83	3.17	3.099	3.55	3.77	3.28
6	1.05	1.39	1.319	1.77	0.99	1.30
Filter	0.00	0.00	0.00	0.00	0.00	0.00
TED (µg)	74.84	78.58	77.80	82.76	74.98	77.79
TED µg/dose	7.48	7.86	7.78	8.28	7.50	7.78
TED %	62.37	65.48	64.83	68.97	62.48	64.83
%FPD (nominal)	20.73	22.43	22.07	24.33	21.09	22.13
%FPF (emitted)	33.23	34.25	34.04	35.27	33.76	34.11
MMAD (µm)	3.6	3.6	3.6	3.7	3.6	3.62
GSD	1.9	2.3	1.9	2.1	1.9	2.02

Table 5.19: Aerodynamic particle profile Foradil Aeroliser® following one inhalation at flows of 90 L/min and inhalation volume of 2 L.

Amount deposited on each stage (µg)						
Stage	**Run 1**	**Run 2**	**Run 3**	**Run 4**	**Run 5**	**Mean µg**
Induction port	10.36	10.24	10.16	10.48	10.59	10.37
-2	0.87	0.75	0.67	0.99	1.10	0.88
-1	1.39	1.27	1.19	1.51	1.62	1.40
0	2.29	2.17	2.09	2.41	2.52	2.30
1	3.92	3.80	3.72	4.04	4.15	3.93
2	2.65	2.53	2.45	2.77	2.88	2.66
3	2.49	2.37	2.29	2.61	2.72	2.50
4	1.28	1.16	1.08	1.40	1.51	1.29
5	0.89	0.77	0.69	0.71	0.12	0.64
Filter	0.00	0.00	0.00	0.00	0.00	0.00
TED (µg)	39.97	38.65	37.77	40.99	41.50	39.78
TED µg/dose	4.00	3.87	3.78	4.10	4.15	3.98
TED %	33.31	32.21	31.48	34.16	34.58	33.15
%FPD (nominal)	11.27	10.67	10.27	11.62	11.58	11.08
%FPF (emitted)	33.83	33.12	32.62	34.01	33.49	33.41
MMAD (µm)	3.60	3.60	3.70	3.70	3.70	3.66
GSD	2.10	2.10	2.10	2.30	2.30	2.18

Table 5.20: Aerodynamic particle profile Foradil Aeroliser® following two inhalations at flows of 90 L/min and inhalation volume of 2 L.

Amount deposited on each stage (µg)						
Stage	**Run 1**	**Run 2**	**Run 3**	**Run 4**	**Run 5**	**Mean µg**
Induction port	16.01	16.35	16.279	16.73	15.95	16.26
-2	2.52	2.43	2.63	2.86	2.21	2.53
-1	4.92	4.83	5.03	5.26	4.61	4.93
0	5.44	5.35	5.55	5.78	5.13	5.45
1	7.81	7.72	7.92	7.15	7.50	7.62
2	5.34	6.25	5.45	6.68	6.03	5.95
3	4.98	4.89	5.09	5.32	4.67	4.99
4	3.83	3.74	3.94	3.17	3.52	3.64
5	1.85	1.76	1.96	2.19	1.54	1.86
Filter	0.37	0.03	0.48	0.71	0.06	0.33
TED (µg)	81.83	81.47	83.15	84.91	79.07	82.09
TED µg/dose	8.18	8.15	8.32	8.49	7.91	8.21
TED %	68.19	67.89	69.29	70.76	65.90	68.41
%FPD (nominal)	24.68	24.77	25.33	25.83	23.69	24.86
%FPF (emitted)	36.20	36.48	36.55	36.51	35.95	36.34
MMAD (µm)	3.1	3.1	3.1	3.2	3.1	3.12
GSD	2.1	2.1	2	1.9	1.8	1.98

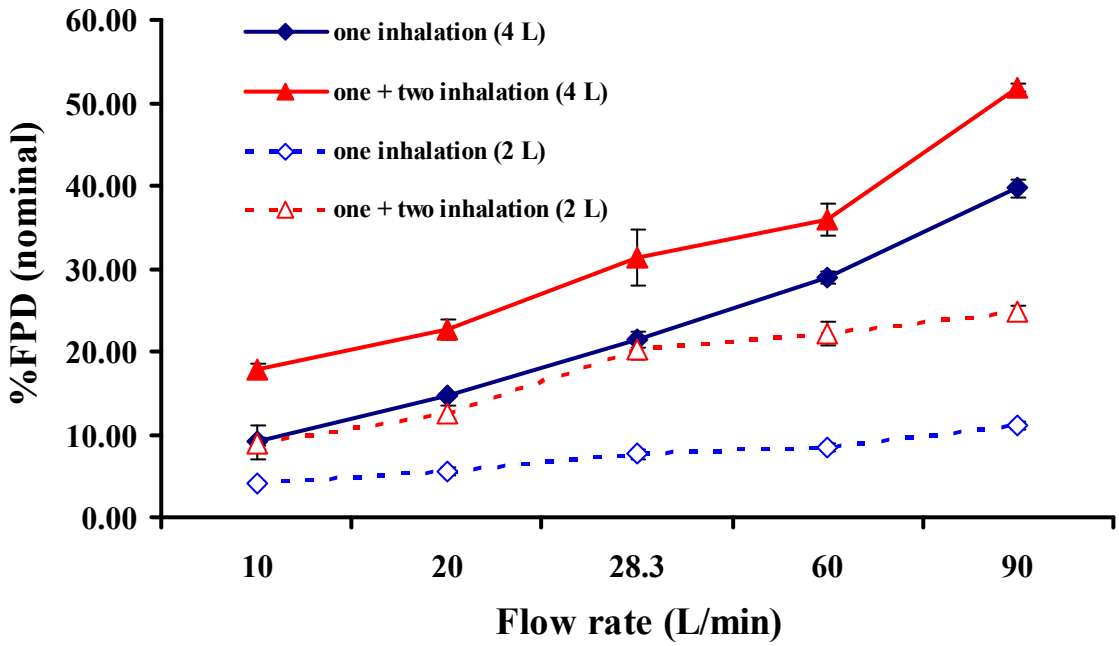

Figure 5.3: The effect of varying inhalation flows (10, 20, 28.3, 60, 90 L/min) on the mean nominal (%) fine particle dose from Aeroliser following one and two inhalations at inhalation volumes of 4 L and 2L.

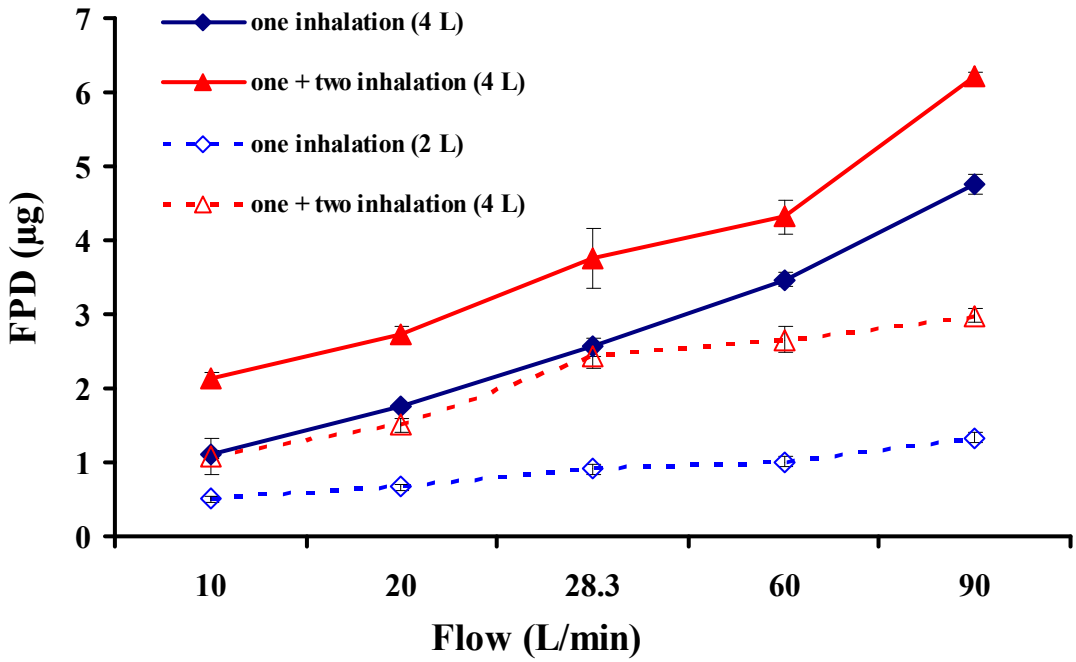

Figure 5.4: The effect of varying inhalation flows (10, 20, 28.3, 60, 90 L/min) on the mean fine particle dose (µg) from Aeroliser following one and two inhalations at inhalation volumes of 4 L and 2L.

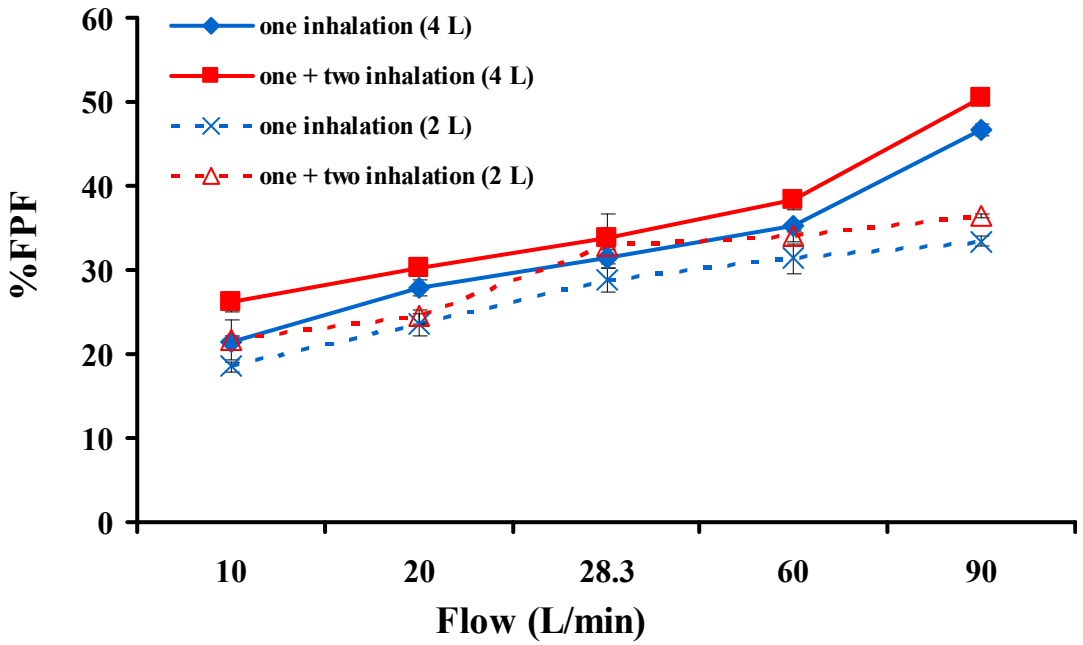

Figure 5.5: The effect of varying inhalation flows of (10, 20, 28.3, 60, 90 L/min) on the mean emitted (%) fine particle fraction from Aeroliser following one and two inhalations at inhalation volumes of 4 L and 2L.

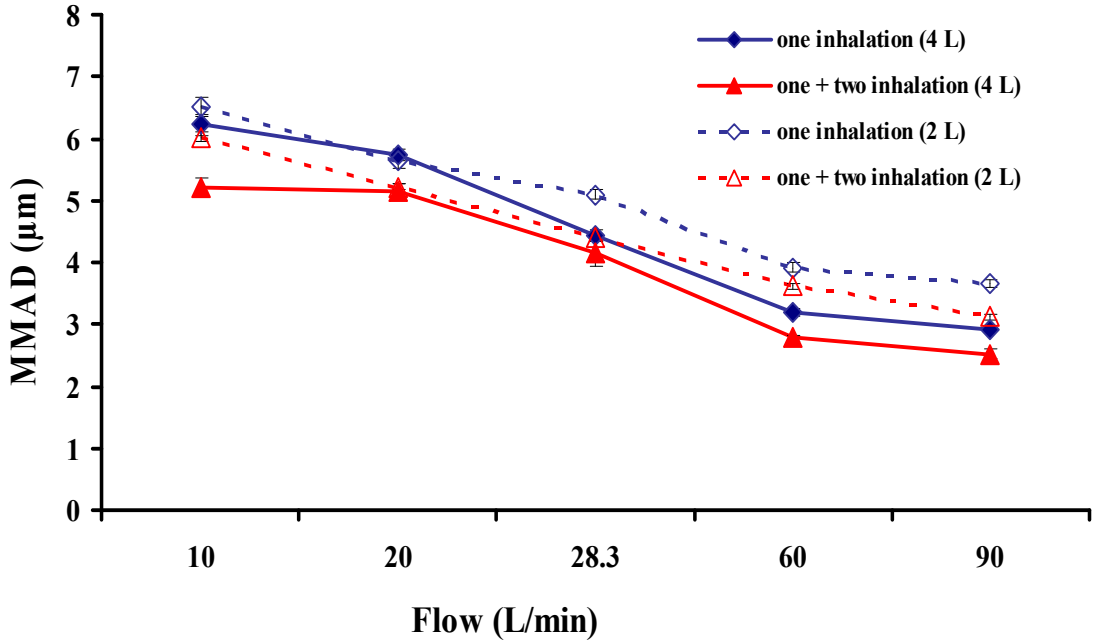

Figure 5.6: The effect of varying inhalation flows (10, 20, 28.3, 60, 90 L/min) on the mean median aerodynamic diameter (MMAD) Aeroliser one and two inhalations at inhalation volumes of 4 L and 2L.

5.3.1.2 The effect of inhalation flow rate on particle size distribution and deposition from Easyhaler®.

Fine particle amount produced from Easyhaler® were also shown to be highly dependent on inhalation flow rate. The nominal fine particle dose expressed as % nominal for formoterol at varying flows of 10, 20, 28.3, 40, and 60 L/min were 19.03%, 27.09%, 36.89%, 49.71%, 49.25% at the inhalation volume of 4 L (one inhalation) and 9.14%, 15.44%, 21.02%, 29.41%, 29.14% at the inhalation volume of 2 L (one inhalation), respectively (Figures 5.7-5.8 and Tables 5.21-5.40). The increase in inhalation flow rates from 10 to 40 L/min showed significant ($p<0.01$-$p<0.001$) increase in the mean nominal fine particle dose using inhalation volumes of 4 L and 2 L. At higher flow rate of 60 L/min no significant differences in the fine particle dose were observed (Figures 5.7-5.8 and Tables 5.21-5.40). The MMAD significantly ($p<0.001$) decreased with increase in the inhalation flow rate from 10 to 60 L/min using inhalation volumes of 4 L and 2 L (Figure 5.10 and Tables 5.21-5.40). There was also significant ($p<0.001$) differences in the fine particle dose achieved using a inhalation volume of 4 L as compared to 2 L with much higher fine particle amount achieved at inhalation volumes of 4 L (Figures 5.7-5.9 and Tables 5.21-5.40 and 5.64). The mean average nominal fine particle dose from Easyhaler® showed no significant differences between one and two inhalations at lower inhalation flow rates of 10, 20, 28.3 L/min using inhalation volumes of 4 L and 2L. Higher inhalation flow rates of 40 and 60 L/min showed significant ($p<0.05$) increased mean nominal fine particle dose at both 4 L and 2 L inhalation volumes compared to one inhalation (Figures 5.7-5.8 and Tables 5.21-5.40).

Table 5.21: Aerodynamic particle profile from Easyhale®r following one inhalation at flows of 10 L/min and inhalation volume of 4 L.

Amount deposited on each stage (µg)						
Stage	**Run 1**	**Run 2**	**Run 3**	**Run 4**	**Run 5**	**Mean µg**
Induction port	15.34	15.00	15.12	16.10	15.76	15.46
-1	6.23	5.89	6.01	6.99	6.65	6.35
0	4.01	3.67	3.79	4.77	4.43	4.13
1	1.34	1.00	1.12	2.10	1.76	1.46
2	0.34	0.00	0.12	1.10	0.76	0.46
3	8.34	8.00	8.12	9.10	8.76	8.46
4	6.87	6.53	6.65	7.63	7.29	6.99
5	3.82	3.48	3.60	4.58	4.24	3.94
6	1.65	1.31	1.43	2.41	2.07	1.77
Filter	0.00	0.00	0.00	0.00	0.00	0.00
TED (µg)	71.39	67.65	68.97	79.75	76.01	72.75
TED µg/dose	7.14	6.77	6.90	7.98	7.60	7.28
TED %	59.49	56.38	57.48	66.46	63.34	60.63
%FPD (nominal)	17.52	16.93	17.53	22.43	20.73	19.03
%FPF (emitted)	29.44	30.04	30.51	33.76	32.73	31.29
MMAD (µm)	6.00	6.10	6.15	4.90	4.90	5.61
GSD	2.50	2.10	2.60	2.50	2.40	2.42

Table 5.22: Aerodynamic particle profile from Easyhaler® following two inhalations at flows of 10 L/min and inhalation volume of 4 L.

Amount deposited on each stage (µg)						
Stage	**Run 1**	**Run 2**	**Run 3**	**Run 4**	**Run 5**	**Mean µg**
Induction port	15.46	15.94	15.77	16	15.34	15.70
-1	6.35	6.83	6.66	6.89	6.23	6.59
0	4.13	4.61	4.44	4.67	4.01	4.37
1	1.46	1.94	1.77	1.9	1.34	1.68
2	0.46	0.94	0.77	0.98	0.34	0.70
3	8.46	8.94	8.77	9.06	8.34	8.71
4	6.99	7.47	7.3	7.53	6.87	7.23
5	3.94	4.42	4.25	4.48	3.82	4.18
6	1.77	2.25	2.08	2.31	1.65	2.01
Filter	0.00	0.00	0.00	0	0.00	0.00
TED (µg)	72.71	77.99	76.12	78.59	71.39	75.36
TED µg/dose	7.27	7.80	7.61	7.86	7.14	7.54
TED %	60.59	64.99	63.43	65.49	59.49	62.80
%FPD (nominal)	19.23	21.63	20.78	21.88	18.63	20.43
%FPF (emitted)	31.74	33.29	32.76	33.41	31.32	32.51
MMAD (µm)	6.4	6.4	6.4	6.5	6.4	6.42
GSD	2.2	2.3	2.3	2.0	2.2	2.20

Table 5.23: Aerodynamic particle profile from Easyhaler® following one inhalation at flows of 20 L/min and inhalation volume of 4 L.

Amount deposited on each stage (µg)						
Stage	**Run 1**	**Run 2**	**Run 3**	**Run 4**	**Run 5**	**Mean µg**
Induction port	15.46	15.69	15.60	15.99	15.71	15.69
-1	6.47	7.94	6.61	7.00	6.72	6.95
0	4.79	5.02	4.93	5.32	5.04	5.02
1	2.59	2.82	2.73	3.12	2.84	2.82
2	1.91	2.14	2.05	2.44	2.16	2.14
3	11.54	11.77	11.68	12.07	11.79	11.77
4	8.32	8.55	8.46	8.85	8.57	8.55
5	4.41	4.64	4.55	4.94	4.66	4.64
6	2.88	3.11	3.02	3.41	3.13	3.11
Filter	0.00	0.00	0.00	0.00	0.00	0.00
TED (µg)	83.18	86.95	84.72	89.01	85.93	85.96
TED µg/dose	8.32	8.70	8.47	8.90	8.59	8.60
TED %	69.32	72.46	70.60	74.18	71.61	71.63
%FPD (nominal)	24.22	27.53	27.08	29.03	27.63	27.09
%FPF (emitted)	34.94	37.99	38.35	39.13	38.58	37.80
MMAD (µm)	4.60	4.40	4.50	4.60	4.40	4.50
GSD	2.50	2.60	2.60	2.50	2.30	2.50

Table 5.24: Aerodynamic particle profile from Easyhaler® following two inhalations at flows of 20 L/min and inhalation volume of 4 L.

Amount deposited on each stage (µg)						
Stage	**Run 1**	**Run 2**	**Run 3**	**Run 4**	**Run 5**	**Mean µg**
Induction port	16.00	16.56	15.36	15.75	15.6	15.85
-1	7.01	7.57	6.37	6.76	6.61	6.86
0	5.33	5.89	4.69	5.08	4.93	5.18
1	3.13	3.69	2.49	2.88	2.73	2.98
2	2.45	3.01	1.81	2.2	2.05	2.30
3	12.08	12.64	11.44	11.83	11.68	11.93
4	8.86	9.42	8.22	8.61	8.46	8.71
5	4.95	5.51	4.31	4.7	4.55	4.80
6	3.42	3.98	2.78	3.17	3.02	3.27
Filter	0.00	0.00	0.00	0.00	0.00	0.00
TED (µg)	89.12	95.28	82.08	86.37	84.72	87.51
TED µg/dose	8.91	9.53	8.21	8.64	8.47	8.75
TED %	74.27	79.40	68.40	71.98	70.60	72.93
%FPD (nominal)	29.08	31.88	25.88	27.83	27.08	28.35
%FPF (emitted)	39.15	40.14	37.83	38.66	38.35	38.83
MMAD (µm)	4.5	4.6	4.7	4.5	4.4	4.54
GSD	2.1	2.3	2.1	2.2	2.2	2.18

Table 5.25: Aerodynamic particle profile from Easyhaler® following one inhalation at flows of 28.3 L/min and inhalation volume of 4 L.

Amount deposited on each stage (µg)						
Stage	**Run 1**	**Run 2**	**Run 3**	**Run 4**	**Run 5**	**Mean µg**
Induction port	15.59	15.93	16.72	16.33	16.45	16.20
-1	7.60	7.94	8.73	8.34	8.46	8.21
0	6.92	7.26	8.05	7.66	7.78	7.53
1	4.72	5.06	5.85	5.46	5.58	5.33
2	2.04	2.17	2.96	2.57	2.69	2.49
3	12.67	12.80	13.59	13.20	13.32	13.12
4	10.45	10.58	11.37	10.98	11.10	10.90
5	8.14	8.27	9.06	8.67	8.79	8.59
6	4.01	4.14	4.93	4.54	4.66	4.46
Filter	0.12	0.18	0.33	0.48	0.56	0.33
TED (µg)	96.33	99.08	107.92	103.78	105.18	102.46
TED µg/dose	9.63	9.91	10.79	10.38	10.52	10.25
TED %	80.28	82.57	89.93	86.48	87.65	85.38
%FPD (nominal)	31.19	36.00	40.08	38.25	38.92	36.89
%FPF (emitted)	38.86	43.60	44.56	44.23	44.40	43.13
MMAD (µm)	3.70	3.80	3.80	3.70	3.80	3.76
GSD	1.90	2.00	2.10	2.20	2.10	2.06

Table 5.26: Aerodynamic particle profile from Easyhaler® following two inhalations at flows of 28.3 L/min and inhalation volume of 4 L.

Amount deposited on each stage (µg)						
Stage	**Run 1**	**Run 2**	**Run 3**	**Run 4**	**Run 5**	**Mean µg**
Induction port	16.05	16.87	16.15	16.32	17	16.48
-1	8.06	8.88	8.16	8.33	9.01	8.49
0	7.38	8.2	7.48	7.65	8.33	7.81
1	5.18	6	5.28	5.45	6.13	5.61
2	2.50	3.11	2.39	2.56	3.24	2.76
3	13.13	13.74	13.02	13.19	13.87	13.39
4	10.91	11.52	10.8	10.97	11.65	11.17
5	8.60	9.21	8.49	8.66	9.34	8.86
6	4.47	5.08	4.36	4.53	5.21	4.73
Filter	0.24	0.11	0.34	0.63	0.51	0.37
TED (µg)	101.51	109.35	101.66	103.82	111.18	105.50
TED µg/dose	10.15	10.94	10.17	10.38	11.12	10.55
TED %	84.59	91.13	84.72	86.52	92.65	87.92
%FPD (nominal)	37.53	40.64	37.23	38.33	41.63	39.07
%FPF (emitted)	44.36	44.60	43.95	44.30	44.93	44.43
MMAD (µm)	3.9	3.8	3.9	3.8	3.8	3.84
GSD	1.9	2.3	2	2.1	2.2	2.10

Table 5.27: Aerodynamic particle profile from Easyhaler® following one inhalation at flows of 40 L/min and inhalation volume of 4 L.

Amount deposited on each stage (µg)						
Stage	**Run 1**	**Run 2**	**Run 3**	**Run 4**	**Run 5**	**Mean µg**
Induction port	13.27	14.38	13.50	11.43	12.30	12.98
-1	6.60	7.71	7.83	7.76	8.63	7.71
0	4.40	5.51	5.63	6.56	6.43	5.71
1	9.72	9.83	7.95	5.88	5.75	7.83
2	8.35	8.46	8.58	8.51	9.38	8.66
3	15.13	14.48	15.60	15.29	14.16	14.93
4	13.82	13.17	12.29	13.98	13.85	13.42
5	8.69	8.04	9.16	9.85	8.72	8.89
6	4.16	5.51	6.63	6.32	6.19	5.76
Filter	0.12	0.18	0.33	0.48	0.56	0.33
TED (µg)	103.64	104.88	107.19	105.51	106.16	105.48
TED µg/dose	10.36	10.49	10.72	10.55	10.62	10.55
TED %	86.37	87.40	89.33	87.93	88.47	87.90
%FPD (nominal)	49.95	49.69	50.28	50.01	48.59	49.71
%FPF (emitted)	57.83	56.86	56.29	56.88	54.93	56.56
MMAD (µm)	3.30	3.10	3.50	3.20	3.30	3.28
GSD	2.10	2.30	2.50	2.70	2.70	2.46

Table 5.28: Aerodynamic particle profile from Easyhaler® following two inhalations at flows of 40 L/min and inhalation volume of 4 L.

Amount deposited on each stage (µg)						
Stage	**Run 1**	**Run 2**	**Run 3**	**Run 4**	**Run 5**	**Mean µg**
Induction port	13.61	14.57	13.69	11.62	12.49	13.20
-1	6.94	7.9	8.02	7.95	8.82	7.93
0	4.74	5.7	5.82	6.75	6.62	5.93
1	10.06	10.02	8.14	6.07	5.94	8.05
2	8.69	8.65	8.77	8.7	9.57	8.88
3	15.47	14.67	15.79	15.48	14.35	15.15
4	14.16	13.36	12.48	14.17	14.04	13.64
5	9.03	8.23	9.35	10.04	8.91	9.11
6	4.50	5.7	6.82	6.51	6.38	5.98
Filter	0.41	0.21	0.98	0.70	0.85	0.63
TED (µg)	107.72	107.04	110.13	108.12	108.84	108.37
TED µg/dose	10.77	10.70	11.01	10.81	10.88	10.84
TED %	89.77	89.20	91.78	90.10	90.70	90.31
%FPD (nominal)	51.93	50.70	51.94	51.39	50.03	51.20
%FPF (emitted)	57.85	56.84	56.60	57.04	55.16	56.70
MMAD (µm)	3.4	3.5	3.7	3.6	3.5	3.54
GSD	2.1	2.1	2.2	2.1	2.2	2.14

Table 5.29: Aerodynamic particle profile from Easyhaler® following one inhalation at flows of 60 L/min and inhalation volume of 4 L.

Amount deposited on each stage (µg)						
Stage	**Run 1**	**Run 2**	**Run 3**	**Run 4**	**Run 5**	**Mean µg**
Induction port	14.36	14.47	15.59	15.52	14.39	14.87
-1	5.69	7.80	6.92	7.85	8.72	7.40
0	4.96	4.07	4.19	4.12	4.99	4.47
1	9.28	9.39	9.51	10.44	9.31	9.59
2	8.91	7.02	8.14	7.07	8.94	8.02
3	15.69	15.04	15.16	14.85	16.72	15.49
4	12.38	11.73	13.85	11.54	13.41	12.58
5	9.25	8.60	7.72	6.41	8.28	8.05
6	5.72	4.07	4.19	3.88	5.75	4.72
Filter	0.57	0.92	0.75	0.67	0.34	0.65
TED (µg)	109.42	104.56	108.09	104.28	114.52	108.17
TED µg/dose	10.94	10.46	10.81	10.43	11.45	10.82
TED %	91.18	87.13	90.08	86.90	95.43	90.15
%FPD (nominal)	51.50	47.31	49.43	45.72	52.29	49.25
%FPF (emitted)	56.48	54.29	54.88	52.61	54.79	54.61
MMAD (µm)	2.70	2.70	2.90	2.70	2.80	2.76
GSD	2.10	2.40	2.20	2.30	2.40	2.28

Table 5.30: Aerodynamic particle profile from Easyhaler® following two inhalations at flows of 60 L/min and inhalation volume of 4 L.

Amount deposited on each stage (µg)						
Stage	**Run 1**	**Run 2**	**Run 3**	**Run 4**	**Run 5**	**Mean µg**
Induction port	6.20	6.04	6.43	5.95	5.70	6.06
-1	5.47	5.31	5.70	5.22	4.97	5.33
0	9.79	9.63	10.02	9.54	9.29	9.65
1	9.42	9.26	9.65	9.17	8.92	9.28
2	16.20	16.04	16.43	15.95	15.70	16.06
3	12.89	12.73	13.12	12.64	12.39	12.75
4	9.76	9.60	9.99	9.51	9.26	9.62
5	6.23	6.07	6.46	5.98	5.73	6.09
6	0.45	0.67	0.60	0.31	0.84	0.57
Filter	0.45	0.67	0.60	0.31	0.84	0.57
TED (µg)	114.91	113.37	117.59	112.02	109.80	113.54
TED µg/dose	11.49	11.34	11.76	11.20	10.98	11.35
TED %	95.76	94.48	97.99	93.35	91.50	94.62
%FPD (nominal)	53.95	53.33	55.23	52.58	51.78	53.37
%FPF (emitted)	56.34	56.45	56.36	56.33	56.58	56.41
MMAD (µm)	2.7	2.6	2.5	2.5	2.4	2.54
GSD	2.1	2.1	2	2.2	2.1	2.10

Table 5.31: Aerodynamic particle profile from Easyhaler® following one inhalation at flows of 10 L/min and inhalation volume of 2 L.

Amount deposited on each stage (µg)						
Stage	**Run 1**	**Run 2**	**Run 3**	**Run 4**	**Run 5**	**Mean µg**
Induction port	10.96	10.71	10.80	11.50	11.26	11.05
-1	4.45	4.21	4.29	4.99	4.75	4.54
0	2.86	2.62	2.71	3.41	3.16	2.95
1	0.64	0.48	0.53	1.00	0.84	0.70
2	0.17	0.35	0.64	0.55	0.38	0.42
3	4.14	3.97	4.03	4.51	4.35	4.20
4	3.41	3.24	3.30	3.78	3.62	3.47
5	1.89	1.73	1.79	2.27	2.10	1.96
6	0.28	0.37	0.29	0.50	0.33	0.36
Filter	0.00	0.00	0.00	0.00	0.00	0.00
TED (µg)	45.55	43.94	44.82	50.35	48.13	46.56
TED µg/dose	4.56	4.39	4.48	5.04	4.81	4.66
TED %	37.96	36.62	37.35	41.96	40.11	38.80
%FPD (nominal)	8.24	8.44	8.82	10.51	9.68	9.14
%FPF (emitted)	21.72	23.06	23.61	25.06	24.12	23.51
MMAD (µm)	6.40	6.40	6.40	6.50	6.60	6.46
GSD	2.40	2.20	2.00	2.10	2.20	2.18

Table 5.32: Aerodynamic particle profile from Easyhaler® following two inhalations at flows of 10 L/min and inhalation volume of 2 L.

Amount deposited on each stage (µg)						
Stage	**Run 1**	**Run 2**	**Run 3**	**Run 4**	**Run 5**	**Mean µg**
Induction port	11.04	11.39	11.26	11.43	10.96	11.22
-1	4.54	4.88	4.76	4.92	4.45	4.71
0	2.95	3.29	3.17	3.34	2.86	3.12
1	0.70	0.92	0.84	0.90	0.64	0.80
2	0.23	0.47	0.38	0.49	0.17	0.35
3	4.20	4.43	4.35	4.49	4.14	4.32
4	3.47	3.71	3.62	3.74	3.41	3.59
5	1.95	2.19	2.11	2.22	1.89	2.07
6	0.88	1.12	1.03	1.15	0.82	1.00
Filter	0.00	0.00	0.00	0.00	0.00	0.00
TED (µg)	46.87	50.00	48.89	50.37	46.09	48.44
TED µg/dose	4.69	5.00	4.89	5.04	4.61	4.84
TED %	39.06	41.67	40.74	41.97	38.41	40.37
%FPD (nominal)	9.52	10.70	10.28	10.82	9.22	10.11
%FPF (emitted)	24.36	25.68	25.23	25.79	24.01	25.01
MMAD (µm)	6.4	6.2	6.4	6.3	6.2	6.30
GSD	1.8	1.7	1.8	1.9	1.8	1.80

Table 5.33: Aerodynamic particle profile from Easyhaler® following one inhalation at flows of 20 L/min and inhalation volume of 2 L.

Amount deposited on each stage (µg)						
Stage	**Run 1**	**Run 2**	**Run 3**	**Run 4**	**Run 5**	**Mean µg**
Induction port	11.45	11.62	11.56	11.84	11.64	11.62
-1	4.79	5.88	4.90	5.19	4.98	5.15
0	3.55	3.72	3.65	3.94	3.73	3.72
1	1.48	1.61	1.56	1.78	1.62	1.61
2	1.09	1.22	1.17	1.39	1.23	1.22
3	6.58	6.71	6.66	6.88	6.72	6.71
4	4.74	4.87	4.82	5.04	4.88	4.87
5	2.51	2.64	2.59	2.81	2.66	2.64
6	1.64	1.77	1.72	1.94	1.78	1.77
Filter	0.00	0.00	0.00	0.00	0.00	0.00
TED (µg)	56.20	58.76	57.20	59.98	57.99	58.03
TED µg/dose	5.62	5.88	5.72	6.00	5.80	5.80
TED %	46.84	48.97	47.67	49.98	48.32	48.36
%FPD (nominal)	13.80	15.68	15.43	16.54	15.74	15.44
%FPF (emitted)	29.46	32.03	32.36	33.09	32.58	31.90
MMAD (µm)	4.60	4.50	4.40	4.60	4.60	4.54
GSD	2.00	1.90	1.80	2.00	2.00	1.94

Table 5.34: Aerodynamic particle profile from Easyhaler® following two inhalations at flows of 20 L/min and inhalation volume of 2 L.

Amount deposited on each stage (µg)						
Stage	**Run 1**	**Run 2**	**Run 3**	**Run 4**	**Run 5**	**Mean µg**
Induction port	11.85	12.27	11.38	11.67	11.56	11.74
-1	5.19	5.61	4.72	5.01	4.90	5.08
0	3.95	4.36	3.47	3.76	3.65	3.84
1	1.78	2.10	1.42	1.64	1.56	1.70
2	1.40	1.72	1.03	1.25	1.17	1.31
3	6.88	7.20	6.52	6.74	6.66	6.80
4	5.05	5.37	4.68	4.91	4.82	4.97
5	2.82	3.14	2.46	2.68	2.59	2.74
6	1.95	2.27	1.58	1.81	1.72	1.87
Filter	0.00	0.00	0.00	0.00	0.00	0.00
TED (µg)	60.05	64.04	55.49	58.27	57.20	59.01
TED µg/dose	6.01	6.40	5.55	5.83	5.72	5.90
TED %	50.04	53.37	46.24	48.56	47.67	49.18
%FPD (nominal)	16.57	18.16	14.74	15.85	15.43	16.15
%FPF (emitted)	33.11	34.03	31.88	32.65	32.36	32.81
MMAD (µm)	4.3	4.4	4.5	4.6	4.4	4.44
GSD	2.1	1.8	1.9	1.8	1.9	1.90

Table 5.35: Aerodynamic particle profile from Easyhaler® following one t inhalation at flows of 28.3 L/min and inhalation volume of 2 L.

Amount deposited on each stage (µg)						
Stage	**Run 1**	**Run 2**	**Run 3**	**Run 4**	**Run 5**	**Mean µg**
Induction port	11.55	11.80	12.39	12.10	12.19	12.00
-1	5.63	5.88	6.47	6.18	6.27	6.08
0	5.13	5.38	5.96	5.67	5.76	5.58
1	2.69	2.88	3.33	3.11	3.18	3.04
2	1.16	1.24	1.69	1.46	1.53	1.42
3	7.22	7.29	7.74	7.52	7.59	7.47
4	5.95	6.03	6.48	6.26	6.32	6.21
5	4.64	4.71	5.16	4.94	5.01	4.89
6	2.28	2.36	2.81	2.59	2.66	2.54
Filter	0.07	0.10	0.19	0.27	0.32	0.19
TED (µg)	64.15	66.01	71.72	69.03	69.93	68.17
TED µg/dose	6.42	6.60	7.17	6.90	6.99	6.82
TED %	53.46	55.01	59.77	57.52	58.27	56.81
%FPD (nominal)	17.77	20.51	22.83	21.79	22.17	21.02
%FPF (emitted)	33.25	37.29	38.21	37.89	38.05	36.94
MMAD (µm)	3.70	3.80	3.70	3.60	3.90	3.74
GSD	2.10	2.10	2.10	1.90	2.10	2.06

Table 5.36: Aerodynamic particle profile from Easyhaler® following two inhalations at flows of 28.3 L/min and inhalation volume of 2 L.

Amount deposited on each stage (µg)						
Stage	**Run 1**	**Run 2**	**Run 3**	**Run 4**	**Run 5**	**Mean µg**
Induction port	11.89	12.50	11.96	12.09	12.59	12.21
-1	5.97	6.58	6.04	6.17	6.67	6.29
0	5.47	6.07	5.54	5.67	6.17	5.78
1	2.95	3.42	3.01	3.11	3.49	3.20
2	1.42	1.77	1.36	1.46	1.85	1.57
3	7.48	7.83	7.42	7.52	7.90	7.63
4	6.22	6.56	6.15	6.25	6.64	6.36
5	4.90	5.25	4.84	4.93	5.32	5.05
6	2.55	2.89	2.48	2.58	2.97	2.70
Filter	0.14	0.06	0.19	0.36	0.29	0.21
TED (µg)	67.50	72.66	67.67	69.04	73.82	70.14
TED µg/dose	6.75	7.27	6.77	6.90	7.38	7.01
TED %	56.25	60.55	56.39	57.54	61.51	58.45
%FPD (nominal)	21.38	23.16	21.22	21.84	23.72	22.26
%FPF (emitted)	38.01	38.24	37.62	37.96	38.56	38.08
MMAD (µm)	3.8	3.9	4	3.8	3.8	3.86
GSD	2.2	1.9	2.2	2.1	2.1	2.10

Table 5.37: Aerodynamic particle profile from Easyhaler® following one inhalation at flows of 40 L/min and inhalation volume of 2 L.

Amount deposited on each stage (µg)						
Stage	**Run 1**	**Run 2**	**Run 3**	**Run 4**	**Run 5**	**Mean µg**
Induction port	10.21	11.06	10.38	8.79	9.46	9.98
-1	5.08	5.93	6.02	5.97	6.64	5.93
0	3.38	4.24	4.33	5.05	4.95	4.39
1	5.75	5.82	4.70	3.48	3.40	4.63
2	4.94	5.01	5.08	5.04	5.55	5.12
3	8.95	8.57	9.23	9.05	8.38	8.84
4	8.18	7.79	7.27	8.27	8.20	7.94
5	5.14	4.76	5.42	5.83	5.16	5.26
6	2.46	3.26	3.92	3.74	3.66	3.41
Filter	0.04	0.08	0.08	0.11	0.15	0.09
TED (µg)	69.08	70.09	71.74	70.51	71.31	70.55
TED µg/dose	6.91	7.01	7.17	7.05	7.13	7.05
TED %	57.57	58.41	59.79	58.76	59.43	58.79
%FPD (nominal)	29.56	29.40	29.75	29.59	28.75	29.41
%FPF (emitted)	51.34	50.34	49.77	50.36	48.38	50.04
MMAD (µm)	3.30	3.30	3.40	3.50	3.20	3.34
GSD	2.00	2.20	1.80	1.90	2.00	1.98

Table 5.38: Aerodynamic particle profile from Easyhaler® following two inhalations at flows of 40 L/min and inhalation volume of 2 L.

Amount deposited on each stage (µg)						
Stage	**Run 1**	**Run 2**	**Run 3**	**Run 4**	**Run 5**	**Mean µg**
Induction port	10.47	11.21	10.53	8.94	9.61	10.15
-1	5.34	6.08	6.17	6.12	6.78	6.10
0	3.65	4.38	4.48	5.19	5.09	4.56
1	5.95	5.93	4.82	3.59	3.51	4.76
2	5.14	5.12	5.19	5.15	5.66	5.25
3	9.15	8.68	9.34	9.16	8.49	8.97
4	8.38	7.91	7.38	8.38	8.31	8.07
5	5.34	4.87	5.53	5.94	5.27	5.39
6	2.66	3.37	4.04	3.85	3.78	3.54
Filter	0.24	0.12	0.58	0.41	0.50	0.37
TED (µg)	71.80	71.54	73.65	72.22	73.07	72.46
TED µg/dose	7.18	7.15	7.37	7.22	7.31	7.25
TED %	59.83	59.62	61.38	60.18	60.89	60.38
%FPD (nominal)	30.73	30.00	30.73	30.41	29.61	30.30
%FPF (emitted)	51.36	50.32	50.08	50.53	48.62	50.18
MMAD (µm)	3.1	3.2	3.2	3.2	3.4	3.22
GSD	2.2	2.1	2.1	2.2	2.1	2.14

Table 5.39: Aerodynamic particle profile from Easyhaler® following one inhalation at flows of 60 L/min and inhalation volume of 2 L.

Amount deposited on each stage (µg)						
Stage	**Run 1**	**Run 2**	**Run 3**	**Run 4**	**Run 5**	**Mean µg**
Induction port	11.05	11.13	11.99	11.94	11.07	11.44
-1	4.38	6.00	5.32	6.04	6.71	5.69
0	3.82	3.13	3.22	3.17	3.84	3.44
1	5.49	5.56	5.63	6.18	5.51	5.67
2	5.27	4.15	4.82	4.18	5.29	4.74
3	9.28	8.90	8.97	8.79	9.89	9.17
4	7.33	6.94	8.20	6.83	7.93	7.44
5	5.47	5.09	4.57	3.79	4.90	4.76
6	3.38	2.41	2.48	2.30	3.40	2.79
Filter	0.34	0.54	0.44	0.40	0.20	0.38
TED (µg)	73.20	70.35	72.62	70.48	76.95	72.72
TED µg/dose	7.32	7.04	7.26	7.05	7.70	7.27
TED %	61.00	58.63	60.51	58.73	64.13	60.60
%FPD (nominal)	30.47	27.99	29.25	27.05	30.94	29.14
%FPF (emitted)	49.96	47.75	48.34	46.06	48.25	48.07
MMAD (µm)	2.70	2.80	2.70	2.60	2.60	2.68
GSD	1.80	1.80	1.70	1.80	1.80	1.78

Table 5.40: Aerodynamic particle profile from Easyhaler® following two inhalations at flows of 60 L/min and inhalation volume of 2 L.

Amount deposited on each stage (µg)						
Stage	**Run 1**	**Run 2**	**Run 3**	**Run 4**	**Run 5**	**Mean µg**
Induction port	11.44	11.32	11.62	11.25	11.05	11.33
-1	4.77	4.65	4.95	4.58	4.38	4.66
0	4.21	4.08	4.38	4.02	3.82	4.10
1	5.79	5.70	5.93	5.64	5.50	5.71
2	5.57	5.48	5.71	5.43	5.28	5.49
3	9.59	9.49	9.72	9.44	9.29	9.51
4	7.63	7.53	7.76	7.48	7.33	7.55
5	5.78	5.68	5.91	5.63	5.48	5.69
6	3.69	3.59	3.82	3.54	3.39	3.61
Filter	0.27	0.40	0.36	0.18	0.50	0.34
TED (µg)	76.90	75.85	78.69	74.97	73.43	75.97
TED µg/dose	7.69	7.58	7.87	7.50	7.34	7.60
TED %	64.08	63.21	65.57	62.47	61.19	63.31
%FPD (nominal)	31.92	31.56	32.68	31.11	30.64	31.58
%FPF (emitted)	49.81	49.93	49.83	49.80	50.06	49.89
MMAD (µm)	2.6	2.5	2.7	2.4	2.5	2.54
GSD	2.2	2.2	2.1	2.2	1.9	2.12

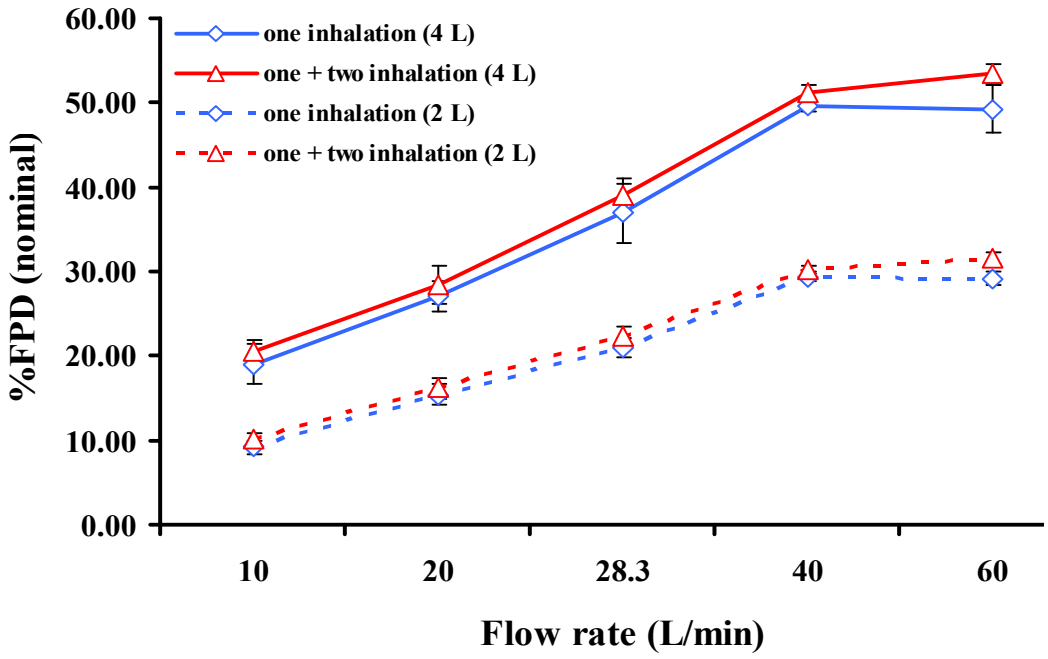

Figure 5.7: The effect of varying inhalation flows (10, 20, 28.3, 40, 60 L/min) on the mean nominal (%) fine particle dose from Easyhaler® following one and two inhalations at inhalation volumes of 4 L and 2L.

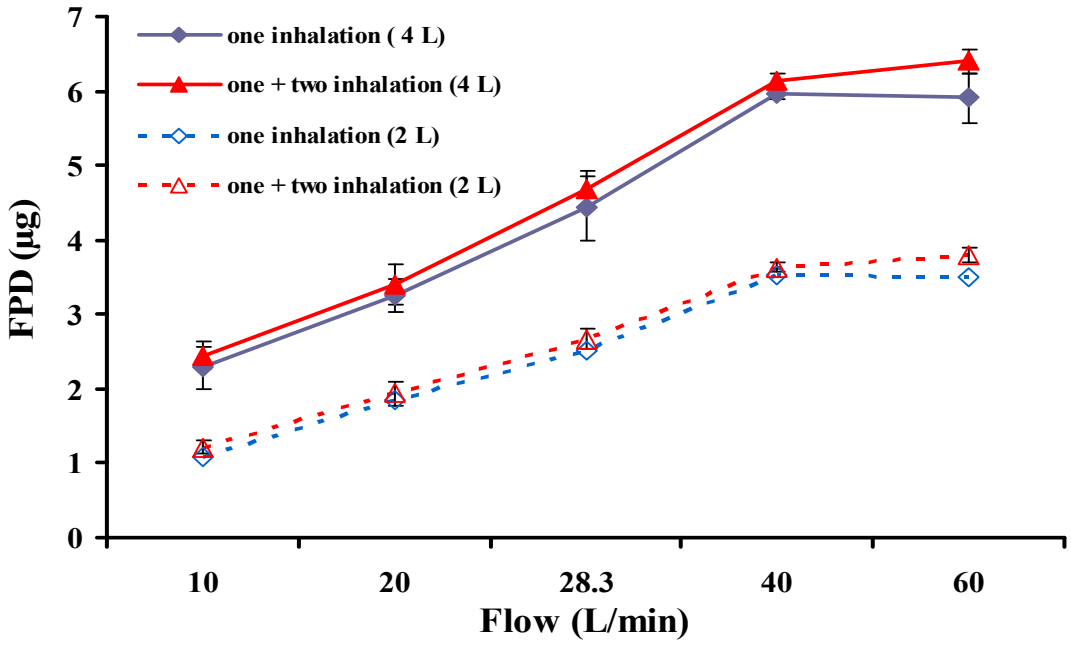

Figure 5.8: The effect of varying inhalation flows (10, 20, 28.3, 40, 60 L/min) on the mean nominal fine particle dose (µg) from Easyhaler® following one and two inhalations at inhalation volumes of 4 L and 2L.

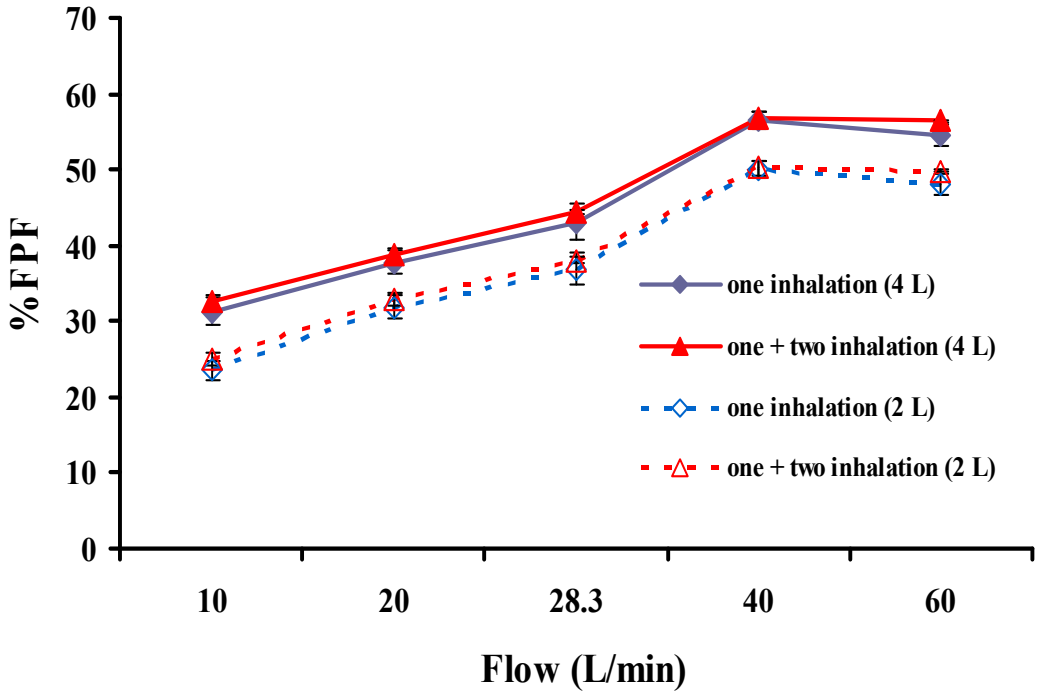

Figure 5.9: The effect of varying inhalation flows of (10, 20, 28.3, 40, 60 L/min) on the mean emitted (%) fine particle fraction from Easyhaler® following one and two inhalations at inhalation volumes of 4 L and 2L.

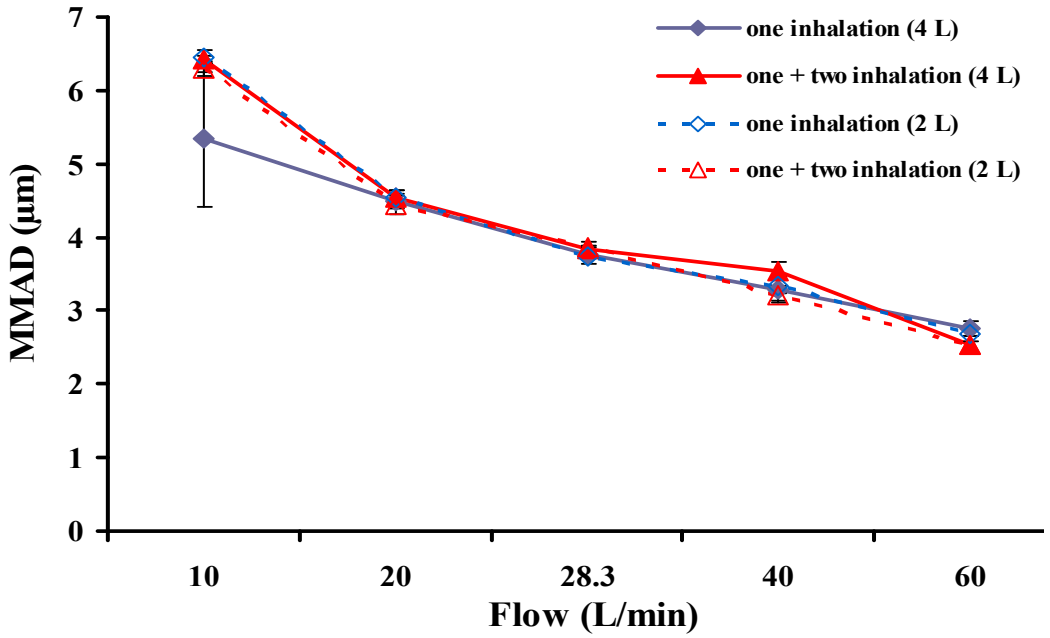

Figure 5.10: The effect of varying inhalation flows (10, 20, 28.3, 40, 60 L/min) on the mean mass aerodynamic diameter (MMAD) Easyhaler® one and two inhalations at inhalation volumes of 4 L and 2L.

5.3.1.3 The effect of inhalation flow rate on particle size distribution and deposition from Oxis Turbuhaler®.

The performance of Oxis Turbuhaler in delivering fine particle dose was also dependent on inhalation flow rate. The nominal fine particle dose for formoterol at varying flows of 10, 20, 28.3, 40, and 60 L/min were 12.87%, 24.51%, 28.25%, 34.61%, 40.53% at the inhalation volume of 4 L (one inhalation) and 8.55%, 15.31%, 21.36%, 19.53%, 22.31% at the inhalation volume of 2 L (one inhalation), respectively (Figure 5.11-5.12 and Tables 5.41-5.60). The increase in inhalation flow rate showed a significant increase in the mean nominal fine particle dose ($p<0.01$- $p<0.001$) at varying inhalation flow rates and inhalation volumes of 4 L and 2 L (Figure 5.11-5.12 and Tables 5.41-5.60). The MMAD significantly ($p<0.001$) decreased with increase in the inhalation

flow rate using inhalation volumes of 4 L and 2 L (Figure 5.14 and Tables 5.41-5.60). Significant increase ($p<0.01$- $p<0.001$) in the fine particle dose were also observed whilst using inhalation volumes of 4 L as opposed to 2 L (Figures 5.11-5.13 and Tables 5.41-5.60 and 5.66). Turbuhaler performance showed no significant differences between one and two inhalations at varying flow rates of 10-60 L/min at 4 L inhalation volumes. A significant ($p<0.05$) increased in the mean emitted fine particle dose was achieved from two inhalation at inhalation flow rates of 10- 60 L/min at 2 L inhalation volumes compared to one inhalation (Figures 5.11-5.12 and Tables 5.41-5.60 and 5.69).

Table 5.41: Aerodynamic particle profile from Turbuhaler® following one inhalation at flows of 10 L/min and inhalation volume of 4 L.

Amount deposited on each stage (µg)						
Stage	**Run 1**	**Run 2**	**Run 3**	**Run 4**	**Run 5**	**Mean µg**
Induction port	14.12	10.80	11.36	13.32	14.99	12.92
-1	1.19	2.01	1.58	1.93	2.06	1.75
0	1.03	1.78	1.48	2.34	1.55	1.64
1	1.34	3.88	3.44	2.74	2.17	2.71
2	2.64	3.76	4.24	3.69	4.43	3.75
3	5.58	4.85	6.28	4.03	3.07	4.76
4	3.52	2.53	2.80	3.29	2.37	2.90
5	0.97	1.04	1.00	1.03	0.68	0.94
6	0.30	0.27	0.06	0.26	0.29	0.24
Filter	0.00	0.00	0.00	0.00	0.00	0.00
TED (µg)	52.21	53.54	53.08	52.76	52.63	52.85
TED µg/dose	5.22	5.35	5.31	5.28	5.26	5.28
TED %	43.51	44.61	44.24	43.97	43.86	44.04
%FPD (nominal)	10.83	15.38	14.83	12.5	10.83	12.87
%FPF (emitted)	24.89	30.50	33.58	28.51	24.69	28.43
MMAD (µm)	6.40	6.40	6.50	6.80	6.50	6.52
GSD	2.00	1.80	2.00	1.80	2.00	1.92

Table 5.42: Aerodynamic particle profile from Turbuhaler® following two inhalations at flows of 10 L/min and inhalation volume of 4 L.

Amount deposited on each stage (µg)						
Stage	**Run 1**	**Run 2**	**Run 3**	**Run 4**	**Run 5**	**Mean µg**
Induction port	14.40	11.01	11.59	13.58	15.29	13.18
-1	1.23	2.09	1.64	2.01	2.14	1.82
0	1.07	1.85	1.53	2.43	1.62	1.70
1	1.39	4.03	3.58	2.85	2.25	2.82
2	2.74	3.91	4.41	3.84	4.60	3.90
3	5.80	5.05	6.53	4.19	3.19	4.95
4	3.66	2.63	2.91	3.43	2.46	3.02
5	1.00	1.08	1.04	1.07	0.71	0.98
6	0.31	0.28	0.06	0.27	0.30	0.24
Filter	0.00	0.00	0.00	0.00	0.00	0.00
TED (µg)	53.59	55.01	54.56	54.20	54.02	54.28
TED µg/dose	5.36	5.50	5.46	5.42	5.40	5.43
TED %	44.66	45.84	45.47	45.17	45.02	45.23
%FPD (nominal)	11.83	14.16	15.41	13.00	11.25	13.13
%FPF (emitted)	27.82	30.87	33.97	28.86	25.02	29.31
MMAD (µm)	6.4	6.4	6.4	6.5	6.4	6.42
GSD	1.9	2	1.9	2.0	2	1.96

Table 5.43: Aerodynamic particle profile from Turbuhaler® following one inhalation at flows of 20 L/min and inhalation volume of 4 L.

Amount deposited on each stage (µg)						
Stage	**Run 1**	**Run 2**	**Run 3**	**Run 4**	**Run 5**	**Mean µg**
Induction port	17.33	14.01	14.57	16.53	18.20	16.13
-1	1.36	2.31	1.81	2.22	2.37	2.01
0	2.28	3.96	3.28	5.20	3.45	3.63
1	4.55	7.09	6.65	5.95	5.38	5.92
2	5.85	6.97	7.45	6.90	7.64	6.96
3	8.79	8.06	9.49	7.24	6.28	7.97
4	6.73	5.74	6.01	6.50	5.58	6.11
5	2.76	2.97	2.85	2.93	1.96	2.69
6	0.85	0.76	0.18	0.75	0.82	0.67
Filter	0.00	0.00	0.00	0.00	0.00	0.00
TED (µg)	67.63	70.08	68.73	69.94	68.29	68.93
TED µg/dose	6.76	7.01	6.87	6.99	6.83	6.89
TED %	56.36	58.40	57.27	58.28	56.91	57.44
%FPD (nominal)	20.83	26.33	27.16	25.25	23	24.51
%FPF (emitted)	36.92	45.08	47.48	43.29	40.48	42.65
MMAD (µm)	4.60	4.60	4.50	4.60	4.50	4.56
GSD	1.60	1.70	1.80	1.80	1.90	1.76

Table 5.44: Aerodynamic particle profile from Turbuhaler® following two inhalations at flows of 20 L/min and inhalation volume of 4 L.

Amount deposited on each stage (µg)						
Stage	**Run 1**	**Run 2**	**Run 3**	**Run 4**	**Run 5**	**Mean µg**
Induction port	18.37	14.85	15.74	17.52	19.29	17.15
-1	1.44	2.45	1.96	2.35	2.51	2.14
0	2.42	4.19	3.54	5.51	3.66	3.86
1	4.82	7.51	7.18	6.31	5.70	6.30
2	5.79	6.90	6.86	6.35	7.03	6.58
3	8.70	7.98	8.73	6.66	5.78	7.57
4	6.66	5.68	5.53	5.98	5.13	5.80
5	2.73	2.94	2.62	2.70	1.80	2.56
6	0.84	0.76	0.16	0.69	0.76	0.64
Filter	0.00	0.00	0.00	0.00	0.00	0.00
TED (µg)	69.94	72.57	70.07	70.73	69.27	70.52
TED µg/dose	6.99	7.26	7.01	7.07	6.93	7.05
TED %	58.28	60.47	58.39	58.94	57.72	58.76
%FPD (nominal)	24.58	26.5	25.91	23.91	21.83	24.55
%FPF (emitted)	42.24	43.78	44.36	40.56	37.80	41.75
MMAD (µm)	4.5	4.6	4.5	4.4	4.4	4.48
GSD	1.8	1.9	1.8	1.7	1.8	1.80

Table 5.45: Aerodynamic particle profile from Turbuhaler® following one inhalation at flows of 28.3 L/min and inhalation volume of 4 L.

Amount deposited on each stage (µg)						
Stage	**Run 1**	**Run 2**	**Run 3**	**Run 4**	**Run 5**	**Mean µg**
Induction port	17.98	16.75	16.35	18.16	18.79	17.61
-1	1.27	2.15	1.69	2.06	2.20	1.87
0	2.17	3.77	3.12	4.95	3.29	3.46
1	4.33	6.75	6.33	5.67	5.12	5.64
2	6.52	6.64	7.10	6.57	7.27	6.82
3	10.99	10.08	11.86	9.05	9.10	10.22
4	8.41	7.18	7.51	8.13	8.22	7.89
5	3.45	3.71	3.57	3.66	2.44	3.37
6	1.06	0.95	0.22	0.94	1.03	0.84
Filter	0.00	0.00	0.00	0.00	0.00	0.00
TED (µg)	74.96	76.83	74.94	79.47	75.76	76.39
TED µg/dose	7.50	7.68	7.49	7.95	7.58	7.64
TED %	62.46	64.02	62.45	66.22	63.13	63.66
%FPD (nominal)	25.33	29.41	30.5	28.33	27.66	28.25
%FPF (emitted)	40.59	45.96	48.82	42.81	43.80	44.40
MMAD (µm)	3.80	3.90	3.80	3.80	3.90	3.84
GSD	1.80	1.90	1.90	1.90	1.80	1.86

Table 5.46: Aerodynamic particle profile from Turbuhaler® following two inhalations at flows of 28.3 L/min and inhalation volume of 4 L.

Amount deposited on each stage (µg)						
Stage	**Run 1**	**Run 2**	**Run 3**	**Run 4**	**Run 5**	**Mean µg**
Induction port	18.37	14.85	15.74	17.52	19.29	17.15
-1	1.44	2.45	1.96	2.35	2.51	2.14
0	2.42	4.19	3.54	5.51	3.66	3.86
1	4.82	7.51	7.18	6.31	5.70	6.30
2	5.79	6.90	6.86	6.35	7.03	6.58
3	8.70	7.98	8.73	6.66	5.78	7.57
4	6.66	5.68	5.53	5.98	5.13	5.80
5	2.73	2.94	2.62	2.70	1.80	2.56
6	0.84	0.76	0.16	0.69	0.76	0.64
Filter	0.00	0.00	0.00	0.00	0.00	0.00
TED (µg)	69.94	72.57	70.07	70.73	69.27	70.52
TED µg/dose	6.99	7.26	7.01	7.07	6.93	7.05
TED %	58.28	60.47	58.39	58.94	57.72	58.76
%FPD (nominal)	30.08	30.58	31.75	29.5	28.75	30.13
%FPF (emitted)	46.81	46.37	49.25	43.21	44.22	45.97
MMAD (µm)	3.7	3.8	3.9	3.8	3.8	3.80
GSD	1.9	1.8	1.9	1.8	1.9	1.86

Table 5.47: Aerodynamic particle profile from Turbuhaler® following one inhalation at flows of 40 L/min and inhalation volume of 4 L.

Amount deposited on each stage (µg)						
Stage	**Run 1**	**Run 2**	**Run 3**	**Run 4**	**Run 5**	**Mean µg**
Induction port	18.16	16.14	14.86	16.51	16.17	16.37
-1	1.51	1.44	1.12	1.37	1.47	1.38
0	3.63	2.51	4.08	3.30	3.53	3.41
1	5.64	5.96	5.64	5.13	5.48	5.57
2	11.36	11.52	10.80	11.43	11.03	11.23
3	12.70	11.06	10.66	11.00	11.09	11.30
4	7.21	6.90	7.07	6.85	7.00	7.01
5	3.26	4.94	4.75	4.89	3.26	4.22
6	2.12	1.91	2.50	1.87	2.06	2.09
Filter	0.00	0.00	0.23	0.14	0.19	0.11
TED (µg)	83.21	79.09	79.61	80.40	80.07	80.48
TED µg/dose	8.32	7.91	7.96	8.04	8.01	8.05
TED %	69.34	65.91	66.34	67.00	66.73	67.07
%FPD (nominal)	35.25	35.25	34.75	34.41	33.41	34.61
%FPF (emitted)	50.83	53.47	52.33	51.37	50.08	51.61
MMAD (µm)	3.30	3.30	3.20	3.30	3.40	3.30
GSD	1.90	1.90	1.80	1.70	1.90	1.84

Table 5.48: Aerodynamic particle profile from Turbuhaler® following two inhalations at flows of 40 L/min and inhalation volume of 4 L.

Amount deposited on each stage (µg)						
Stage	**Run 1**	**Run 2**	**Run 3**	**Run 4**	**Run 5**	**Mean µg**
Induction port	18.37	14.85	15.74	17.52	19.29	17.15
-1	1.44	2.45	1.96	2.35	2.51	2.14
0	2.42	4.19	3.54	5.51	3.66	3.86
1	4.82	7.51	7.18	6.31	5.70	6.30
2	5.79	6.90	6.86	6.35	7.03	6.58
3	8.70	7.98	8.73	6.66	5.78	7.57
4	6.66	5.68	5.53	5.98	5.13	5.80
5	2.73	2.94	2.62	2.70	1.80	2.56
6	0.84	0.76	0.16	0.69	0.76	0.64
Filter	0.00	0.00	0.00	0.00	0.00	0.00
TED (µg)	69.94	72.57	70.07	70.73	69.27	70.52
TED µg/dose	6.99	7.26	7.01	7.07	6.93	7.05
TED %	58.28	60.47	58.39	58.94	57.72	58.76
%FPD (nominal)	37.16	38.08	37.33	37.33	36.16	37.21
%FPF (emitted)	46.81	46.37	49.25	43.21	44.22	45.97
MMAD (µm)	3.7	3.8	3.9	3.8	3.8	3.80
GSD	1.9	1.8	1.9	1.8	1.9	1.86

Table 5.49: Aerodynamic particle profile from Turbuhaler® following one inhalation at flows of 60 L/min and inhalation volume of 4 L.

Amount deposited on each stage (µg)						
Stage	**Run 1**	**Run 2**	**Run 3**	**Run 4**	**Run 5**	**Mean µg**
Induction port	16.04	16.95	16.80	15.81	20.00	17.12
-1	1.81	1.14	0.75	1.08	1.10	1.18
0	4.66	2.31	1.99	2.07	2.74	2.75
1	5.85	5.14	4.99	4.81	4.84	5.12
2	10.09	11.44	13.86	14.70	15.49	13.11
3	15.79	13.22	14.12	16.95	15.91	15.20
4	11.47	11.98	7.71	8.86	9.50	9.91
5	4.43	3.81	3.79	3.24	1.76	3.41
6	2.59	1.02	1.04	0.99	0.46	1.22
Filter	0.85	0.58	0.95	0.71	0.12	0.64
TED (µg)	90.05	85.20	84.51	87.30	91.89	87.79
TED µg/dose	9.00	8.52	8.45	8.73	9.19	8.78
TED %	75.04	71.00	70.43	72.75	76.57	73.16
%FPD (nominal)	42.58	39.33	38.75	41.91	40.08	40.53
%FPF (emitted)	56.71	55.39	54.96	57.58	52.32	55.39
MMAD (µm)	2.70	2.80	2.90	2.70	2.80	2.78
GSD	1.90	1.80	1.90	1.80	1.90	1.86

Table 5.50: Aerodynamic particle profile from Turbuhaler® following two inhalations at flows of 60 L/min and inhalation volume of 4 L.

Amount deposited on each stage (µg)						
Stage	**Run 1**	**Run 2**	**Run 3**	**Run 4**	**Run 5**	**Mean µg**
Induction port	17.65	17.12	15.96	17.39	18.12	17.25
-1	1.99	1.15	0.82	1.19	0.99	1.23
0	4.90	2.33	2.19	2.28	2.47	2.83
1	6.14	5.19	5.49	5.29	4.35	5.29
2	10.59	12.58	15.25	13.17	17.04	13.73
3	16.58	14.54	12.70	18.65	17.50	15.99
4	12.04	13.18	6.94	9.75	10.45	10.47
5	4.65	4.20	3.41	3.56	1.94	3.55
6	2.72	1.12	0.93	1.09	0.51	1.27
Filter	0.90	0.64	0.85	0.78	0.13	0.66
TED (µg)	96.27	89.84	82.15	93.03	91.47	90.55
TED µg/dose	9.63	8.98	8.21	9.30	9.15	9.05
TED %	80.22	74.87	68.46	77.52	76.22	75.46
%FPD (nominal)	44.66	42.83	38	43.58	43.25	42.46
%FPF (emitted)	55.70	57.27	55.48	56.21	56.76	56.28
MMAD (µm)	2.7	2.7	2.9	2.7	2.5	2.70
GSD	2	1.9	1.8	1.9	2.1	1.94

Table 5.51: Aerodynamic particle profile from Turbuhaler® following one inhalation at flows of 10 L/min and inhalation volume of 2 L.

Amount deposited on each stage (µg)						
Stage	**Run 1**	**Run 2**	**Run 3**	**Run 4**	**Run 5**	**Mean µg**
Induction port	11.65	12.82	14.10	12.69	11.42	12.54
-1	0.66	0.41	0.27	0.39	0.40	0.43
0	1.69	0.84	0.72	0.75	0.99	1.00
1	1.55	1.36	1.32	1.27	1.28	1.36
2	2.67	3.38	2.26	2.83	4.10	3.05
3	3.48	3.15	3.39	3.78	3.15	3.39
4	1.98	2.11	2.04	2.35	2.51	2.20
5	0.32	0.39	0.39	0.33	0.18	0.32
6	0.27	0.10	0.11	0.10	0.05	0.13
Filter	0.00	0.00	0.00	0.00	0.00	0.00
TED (µg)	37.01	38.59	40.02	38.39	36.59	38.12
TED µg/dose	3.70	3.86	4.00	3.84	3.66	3.81
TED %	30.84	32.16	33.35	31.99	30.49	31.77
%FPD (nominal)	7.75	8.75	7.91	8.91	9.41	8.55
%FPF (emitted)	23.54	27.21	23.75	27.80	30.83	26.63
MMAD (µm)	7.00	7.00	6.80	7.00	7.00	6.96
GSD	1.80	1.80	1.90	2.00	2.00	1.90

Table 5.52: Aerodynamic particle profile from Turbuhaler® following two inhalations at flows of 10 L/min and inhalation volume of 2 L.

Amount deposited on each stage (µg)						
Stage	**Run 1**	**Run 2**	**Run 3**	**Run 4**	**Run 5**	**Mean µg**
Induction port	11.54	12.69	13.96	12.56	11.31	12.41
-1	0.69	0.43	0.28	0.41	0.42	0.45
0	1.86	0.92	0.79	0.83	1.09	1.10
1	1.70	1.49	1.45	1.40	1.41	1.49
2	2.93	3.71	2.48	3.11	4.50	3.35
3	3.81	3.45	3.71	4.15	3.46	3.72
4	2.17	2.32	2.24	2.57	2.76	2.41
5	0.35	0.43	0.43	0.37	0.20	0.36
6	0.29	0.12	0.12	0.11	0.05	0.14
Filter	0.00	0.00	0.00	0.00	0.00	0.00
TED (µg)	37.19	38.60	39.80	38.42	36.81	38.17
TED µg/dose	3.72	3.86	3.98	3.84	3.68	3.82
TED %	30.99	32.17	33.17	32.01	30.68	31.80
%FPD (nominal)	9.41	9.58	8.66	9.75	10.33	9.55
%FPF (emitted)	30.26	29.85	26.20	30.48	33.62	30.08
MMAD (µm)	6.8	6.9	6.9	6.9	6.9	6.88
GSD	2.1	2	1.8	2.0	1.9	1.96

Table 5.53: Aerodynamic particle profile from Turbuhaler® following one inhalation at flows of 20 L/min and inhalation volume of 2 L.

Amount deposited on each stage (µg)						
Stage	**Run 1**	**Run 2**	**Run 3**	**Run 4**	**Run 5**	**Mean µg**
Induction port	15.33	16.87	18.55	16.70	15.03	16.50
-1	0.89	0.56	0.37	0.53	0.54	0.58
0	2.29	1.13	0.98	1.02	1.34	1.35
1	2.87	2.52	2.45	2.36	2.37	2.51
2	4.95	6.26	4.18	5.25	7.60	5.65
3	6.44	5.83	6.27	7.01	5.84	6.28
4	3.66	3.91	3.78	4.35	4.66	4.07
5	0.73	0.89	0.89	0.76	0.41	0.74
6	0.61	0.24	0.24	0.23	0.11	0.29
Filter	0.00	0.00	0.00	0.00	0.00	0.00
TED (µg)	37.01	38.59	40.02	38.39	36.59	38.12
TED µg/dose	3.70	3.86	4.00	3.84	3.66	3.81
TED %	30.84	32.16	33.35	31.99	30.49	31.77
%FPD (nominal)	13.66	16.41	16.41	16.41	13.66	15.31
%FPF (emitted)	30.04	34.69	30.71	35.33	38.63	33.88
MMAD (µm)	5.00	5.10	5.10	5.10	5.00	5.06
GSD	1.80	1.70	1.80	1.90	1.90	1.82

Table 5.54: Aerodynamic particle profile from Turbuhaler® following two inhalations at flows of 20 L/min and inhalation volume of 2 L.

Amount deposited on each stage (µg)						
Stage	**Run 1**	**Run 2**	**Run 3**	**Run 4**	**Run 5**	**Mean µg**
Induction port	16.10	17.71	19.48	17.53	15.78	17.32
-1	0.93	0.59	0.38	0.56	0.57	0.61
0	2.51	1.24	1.07	1.11	1.47	1.48
1	3.15	2.76	2.68	2.59	2.60	2.76
2	5.43	6.87	4.59	5.75	8.33	6.19
3	7.06	6.39	6.88	7.68	6.41	6.88
4	4.02	4.29	4.15	4.77	5.11	4.47
5	0.80	0.98	0.97	0.83	0.45	0.81
6	0.67	0.26	0.27	0.26	0.12	0.31
Filter	0.00	0.00	0.00	0.00	0.00	0.00
TED (µg)	58.26	60.47	61.78	60.27	58.10	59.78
TED µg/dose	5.83	6.05	6.18	6.03	5.81	5.98
TED %	48.55	50.39	51.48	50.22	48.42	49.81
%FPD (nominal)	17.58	18	16.25	18.25	19.16	17.85
%FPF (emitted)	36.24	35.66	31.63	36.31	39.63	35.89
MMAD (µm)	5	5	5	4	5	4.80
GSD	1.9	1.8	1.9	1.8	1.8	1.84

Table 5.55: Aerodynamic particle profile from Turbuhaler® following one inhalation at flows of 28.3 L/min and inhalation volume of 2 L.

Amount deposited on each stage (µg)						
Stage	**Run 1**	**Run 2**	**Run 3**	**Run 4**	**Run 5**	**Mean µg**
Induction port	14.95	16.38	18.01	16.21	14.59	16.03
-1	0.70	0.75	0.49	0.72	0.73	0.68
0	1.81	1.53	1.32	1.37	1.82	1.57
1	3.20	3.40	3.31	3.19	3.21	3.26
2	10.17	8.46	5.65	7.09	10.27	8.33
3	7.80	7.88	8.47	9.47	7.89	8.30
4	6.21	5.29	5.11	5.87	6.29	5.76
5	0.52	1.21	1.20	1.03	0.56	0.90
6	0.13	0.32	0.33	0.31	0.15	0.25
Filter	0.00	0.00	0.00	0.00	0.00	0.00
TED (µg)	61.77	63.13	63.60	62.99	61.46	62.59
TED µg/dose	6.18	6.31	6.36	6.30	6.15	6.26
TED %	51.48	52.61	53.00	52.49	51.21	52.16
%FPD (nominal)	20.69	22.14	20.06	22.46	23.64	21.80
%FPF (emitted)	40.20	42.08	37.85	42.80	46.15	41.81
MMAD (µm)	4.50	4.60	4.50	4.60	4.50	4.54
GSD	2.00	1.90	2.00	1.80	1.80	1.90

Table 5.56: Aerodynamic particle profile from Turbuhaler® following two inhalations at flows of 28.3 L/min and inhalation volume of 2 L.

Amount deposited on each stage (µg)						
Stage	**Run 1**	**Run 2**	**Run 3**	**Run 4**	**Run 5**	**Mean µg**
Induction port	15.63	17.19	18.91	17.02	15.32	16.82
-1	1.26	0.79	0.52	0.75	0.77	0.82
0	3.39	1.68	1.45	1.51	1.99	2.00
1	4.25	3.73	3.63	3.50	3.52	3.73
2	7.33	9.29	6.20	7.78	11.26	8.37
3	9.54	8.64	9.29	10.38	8.66	9.30
4	5.43	5.80	5.61	6.44	6.90	6.04
5	1.08	1.33	1.32	1.13	0.61	1.09
6	0.90	0.35	0.36	0.35	0.16	0.42
Filter	0.00	0.00	0.00	0.00	0.00	0.00
TED (µg)	65.91	67.61	67.97	67.47	65.95	66.98
TED µg/dose	6.59	6.76	6.80	6.75	6.59	6.70
TED %	54.93	56.34	56.64	56.23	54.96	55.82
%FPD (nominal)	22.75	24.25	22	24.66	25.91	23.91
%FPF (emitted)	43.29	43.10	38.85	43.83	47.18	43.25
MMAD (µm)	4.4	4.4	4.5	4.4	4.4	4.42
GSD	1.8	2	1.9	1.8	1.8	1.86

Table 5.57: Aerodynamic particle profile from Turbuhaler® following one inhalation at flows of 40 L/min and inhalation volume of 2 L.

Amount deposited on each stage (µg)						
Stage	**Run 1**	**Run 2**	**Run 3**	**Run 4**	**Run 5**	**Mean µg**
Induction port	14.45	15.90	17.49	15.74	14.17	15.55
-1	1.28	0.80	0.53	0.76	0.78	0.83
0	3.29	1.63	1.40	1.46	1.93	1.94
1	4.12	3.62	3.52	3.39	3.41	3.61
2	7.11	9.00	6.01	7.54	10.92	8.12
3	9.25	8.38	9.01	10.07	8.40	9.02
4	5.27	5.63	5.44	6.25	6.69	5.86
5	1.51	1.86	1.85	1.58	0.86	1.53
6	1.26	0.50	0.51	0.48	0.22	0.59
Filter	0.00	0.00	0.00	0.00	0.00	0.00
TED (µg)	63.35	64.70	64.88	64.49	62.87	64.06
TED µg/dose	6.34	6.47	6.49	6.45	6.29	6.41
TED %	52.79	53.92	54.06	53.74	52.39	53.38
%FPD (nominal)	23.75	22.16	21.91	24.41	25.41	23.49
%FPF (emitted)	45.03	44.80	40.59	45.45	48.52	44.88
MMAD (µm)	3.90	3.80	3.90	3.90	3.90	3.88
GSD	1.90	1.80	2.00	1.90	1.90	1.90

Table 5.58: Aerodynamic particle profile from Turbuhaler® following two inhalations at flows of 40 L/min and inhalation volume of 2 L.

Amount deposited on each stage (µg)						
Stage	**Run 1**	**Run 2**	**Run 3**	**Run 4**	**Run 5**	**Mean µg**
Induction port	15.18	16.69	18.36	16.53	14.87	16.33
-1	1.34	0.84	0.55	0.80	0.82	0.87
0	3.61	1.78	1.54	1.60	2.12	2.13
1	4.52	3.97	3.86	3.72	3.74	3.96
2	7.80	9.88	6.60	8.27	11.98	8.91
3	10.15	9.19	9.89	11.05	9.21	9.90
4	5.78	6.17	5.97	6.86	7.34	6.42
5	1.66	2.04	2.03	1.73	0.94	1.68
6	1.39	0.54	0.55	0.53	0.25	0.65
Filter	0.00	0.00	0.00	0.00	0.00	0.00
TED (µg)	68.01	69.37	69.42	69.16	67.54	68.70
TED µg/dose	6.80	6.94	6.94	6.92	6.75	6.87
TED %	56.68	57.81	57.85	57.64	56.29	57.25
%FPD (nominal)	26.08	26.5	24.08	26.83	27.91	26.28
%FPF (emitted)	46.01	45.84	41.61	46.50	49.55	45.90
MMAD (µm)	3.8	3.8	3.9	3.8	3.8	3.82
GSD	1.8	1.9	1.7	1.8	1.9	1.82

Table 5.59: Aerodynamic particle profile from Turbuhaler® following one inhalation at flows of 60 L/min and inhalation volume of 2 L.

Amount deposited on each stage (µg)						
Stage	**Run 1**	**Run 2**	**Run 3**	**Run 4**	**Run 5**	**Mean µg**
Induction port	14.03	15.44	16.98	15.28	13.75	15.10
-1	1.36	0.85	0.56	0.81	0.83	0.88
0	3.50	1.73	1.49	1.55	2.06	2.07
1	4.39	3.85	3.74	3.61	3.63	3.84
2	7.56	9.58	6.40	8.02	11.62	8.64
3	9.84	8.91	9.59	10.71	8.93	9.60
4	5.60	5.99	5.79	6.65	7.12	6.23
5	2.32	2.86	2.84	2.43	1.32	2.35
6	1.94	0.76	0.78	0.74	0.35	0.91
Filter	0.64	0.44	0.71	0.53	0.09	0.48
TED (µg)	66.54	67.29	67.44	67.06	64.73	66.61
TED µg/dose	6.65	6.73	6.74	6.71	6.47	6.66
TED %	55.45	56.08	56.20	55.88	53.94	55.51
%FPD (nominal)	26.91	27	22.83	27.25	27.58	22.31
%FPF (emitted)	48.55	48.14	44.25	48.76	51.07	48.15
MMAD (µm)	3.00	3.10	3.00	3.10	3.20	3.08
GSD	1.80	1.80	1.90	1.80	1.80	1.82

Table 5.60: Aerodynamic particle profile from Turbuhaler® following two inhalations at flows of 60 L/min and inhalation volume of 2 L.

Amount deposited on each stage (µg)						
Stage	**Run 1**	**Run 2**	**Run 3**	**Run 4**	**Run 5**	**Mean µg**
Induction port	14.73	16.21	17.83	16.05	14.44	15.85
-1	1.43	0.90	0.59	0.85	0.87	0.93
0	3.84	1.90	1.64	1.71	2.25	2.27
1	4.81	4.23	4.10	3.96	3.98	4.22
2	8.30	10.51	7.02	8.80	12.74	9.47
3	10.80	9.78	10.52	11.75	9.80	10.53
4	6.15	6.57	6.35	7.29	7.81	6.83
5	2.55	3.14	3.12	2.66	1.45	2.58
6	2.13	0.84	0.85	0.82	0.38	1.00
Filter	0.70	0.48	0.78	0.58	0.10	0.53
TED (µg)	71.55	72.26	72.28	72.02	69.62	71.55
TED µg/dose	7.15	7.23	7.23	7.20	6.96	7.15
TED %	59.62	60.22	60.24	60.02	58.02	59.62
%FPD (nominal)	29.5	28.58	27.25	29.91	30.25	29.10
%FPF (emitted)	49.53	49.18	45.28	49.80	52.09	49.18
MMAD (µm)	3	3.1	3.1	3.1	3.1	3.08
GSD	1.8	1.9	1.8	1.9	1.8	1.84

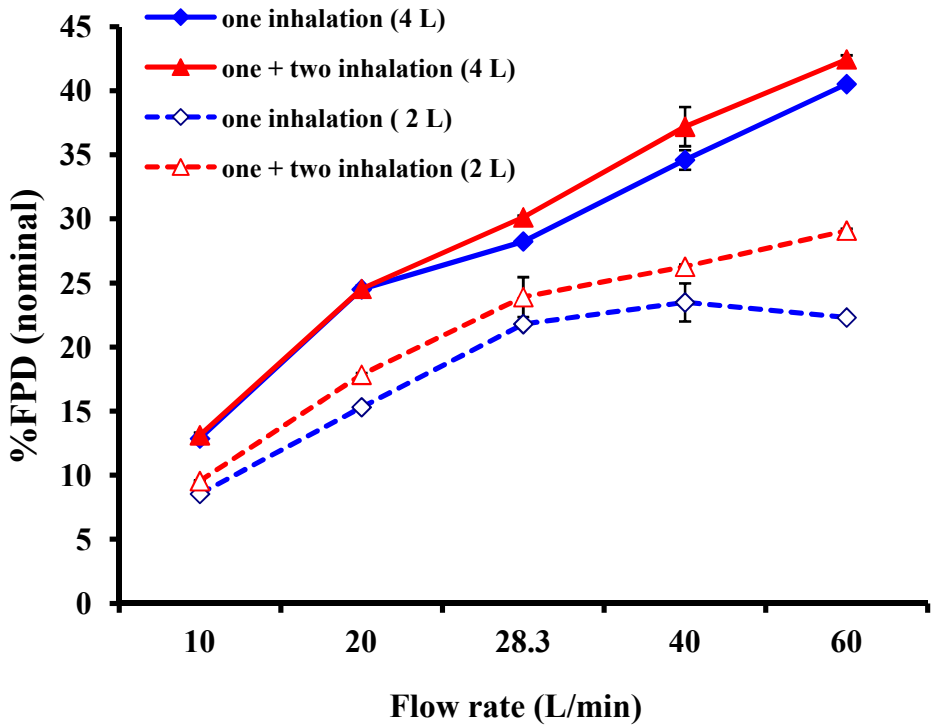

Figure 5.11: The effect of varying inhalation flows (10, 20, 28.3, 40, 60 L/min) on the mean nominal (%) fine particle dose from Turbuhaler® following one and two inhalations at inhalation volumes of 4 L and 2L.

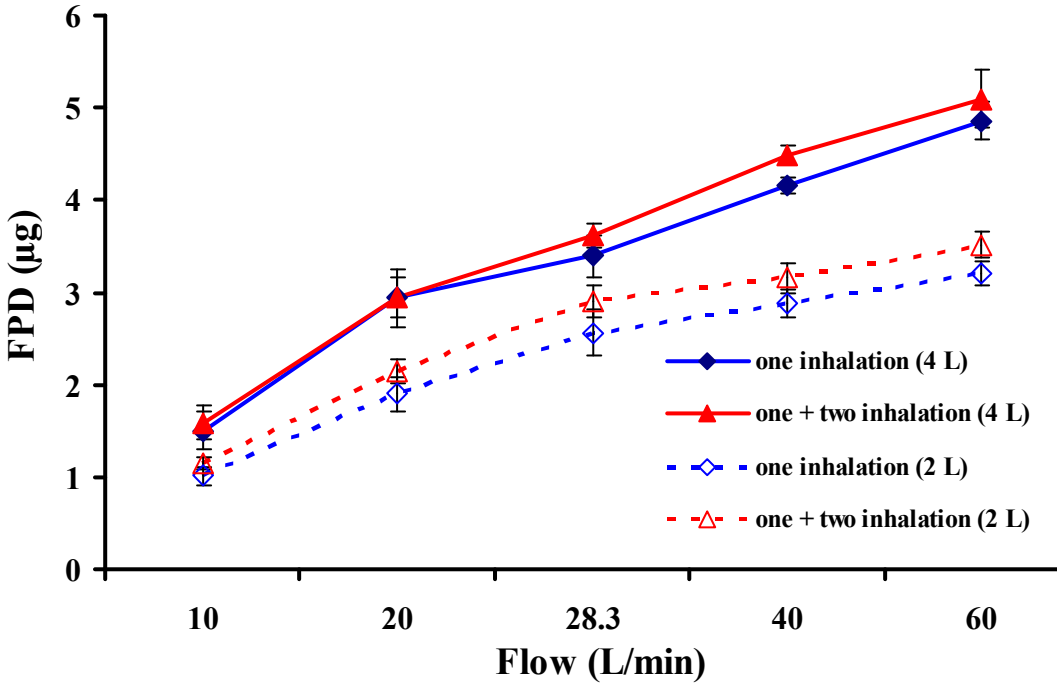

Figure 5.12: The effect of varying inhalation flows (10, 20, 28.3, 40, 60 L/min) on the mean fine particle dose (µg) from Turbuhaler® following one and two inhalations at inhalation volumes of 4 L and 2L.

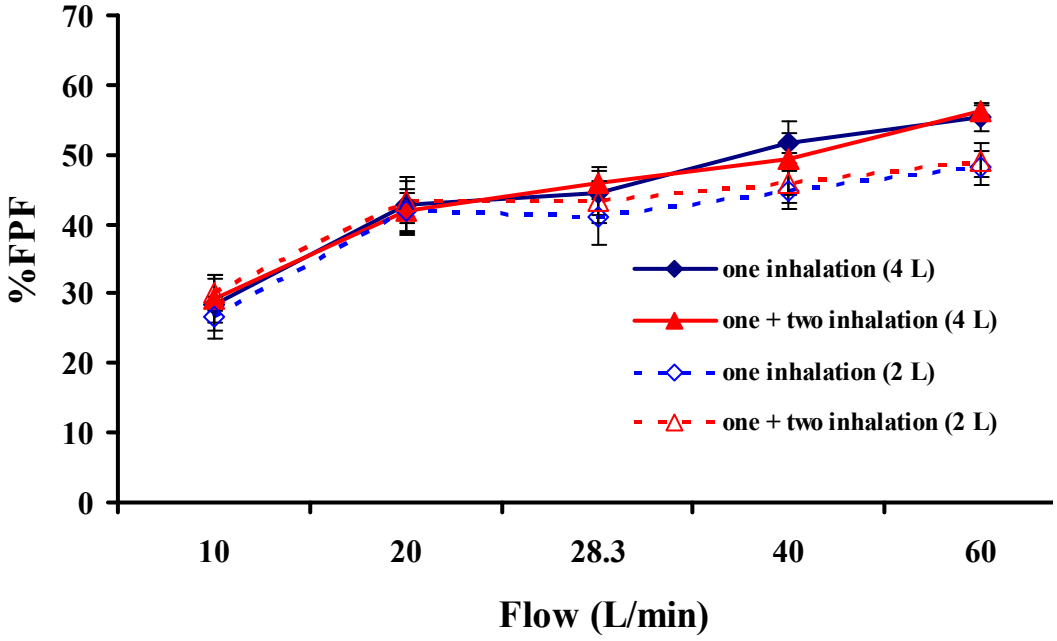

Figure 5.13: The effect of varying inhalation flows (10, 20, 28.3, 40, 60 L/min) on the mean emitted fine particle Fraction (µg) from Turbuhaler® following one and two inhalations at inhalation volumes of 4 L and 2L.

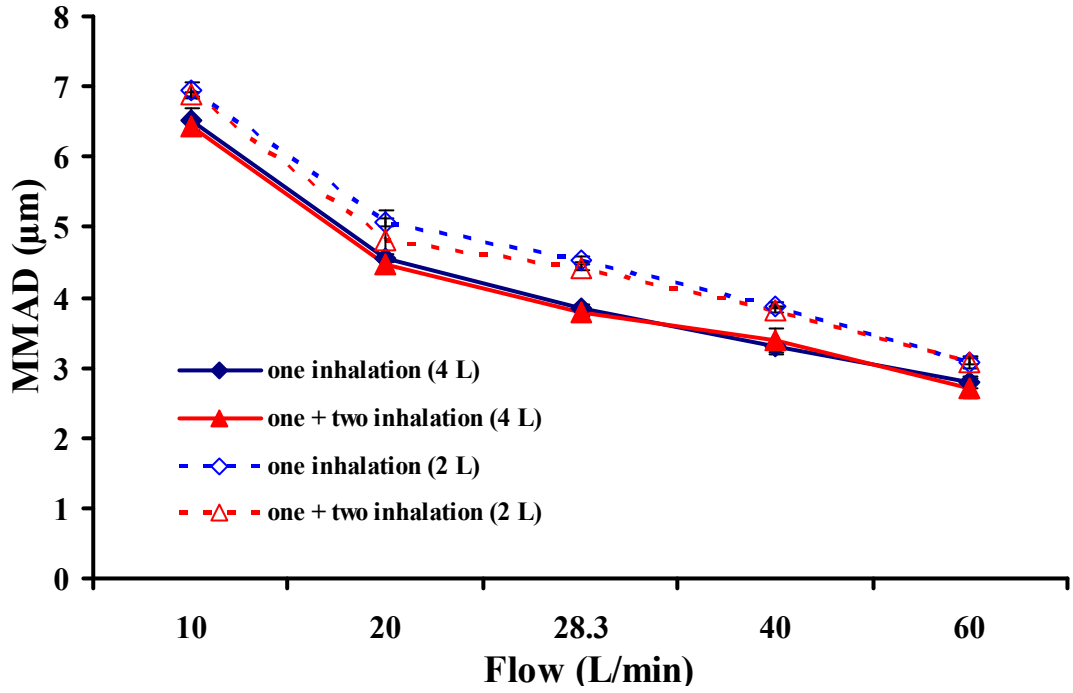

Figure 5.14: The effect of varying inhalation flows (10, 20, 28.3, 40, 60 L/min) on the mean mass aerodynamic diameter (MMAD) Turbuhaler® following one and two inhalations at inhalation volumes of 4 L and 2L.

Table 5.61: Statistical comparison of the % mean nominal fine particle dose and MMAD from the Aeroliser® at varying flow rates of 10, 20, 28.3, 60, 90 L/min using inhalation volumes of 4 L and 2 L. *p<0.05, **p<0.01, ***p<0.001 taken as a significant differences.

Inhalation volume	**4 L**		**2 L**	
Flow rates (L/min)	**% FPD (nominal)**	**MMAD (µm)**	**% FPD (nominal)**	**MMAD (µm)**
10 vs 20 L/min	***p<0.001	***p<0.001	**p<0.01	***p<0.001
10 vs 28.3 L/min	***p<0.001	***p<0.001	***p<0.001	***p<0.001
10 vs 60 L/min	***p<0.001	***p<0.001	***p<0.001	***p<0.001
10 vs 90 L/min	***p<0.001	***p<0.001	***p<0.001	***p<0.001
20 vs 28.3 L/min	***p<0.001	***p<0.001	**p<0.01	***p<0.001
20 vs 60 L/min	***p<0.001	***p<0.001	***p<0.001	***p<0.001

20 vs 90 L/min	***p<0.001	***p<0.001	***p<0.001	***p<0.001
28.3 vs 60 L/min	***p<0.001	***p<0.001	*p<0.05	***p<0.001
28.3 vs 90 L/min	***p<0.001	***p<0.001	***p<0.001	***p<0.001
60 vs 90 L/min	***p<0.001	***p<0.001	***p<0.001	***p<0.001

Table 5.62: Statistical comparison of the % mean nominal fine particle dose from the Aeroliser® using inhalation volumes of 2 L and 4 L at flows of 10, 20, 28.3, 60, 90 L/min. *p<0.05, **p<0.01, ***p<0.001 taken as a significant differences.

	% Mean nominal fine particle dose
4 L vs 2 L 10 L/min	***p<0.001
4 L vs 2 L 20 L/min	***p<0.001
4 L vs 2 L 28.3 L/min	***p<0.001
4 L vs 2 L 60 L/min	***p<0.001
4 L vs 2 L 90 L/min	***p<0.001

Table 5.63: Statistical comparison of the % mean nominal fine particle dose and MMAD from the Easyhaler® at varying flow rates of 10, 20, 28.3, 40, 90 L/min using inhalation volumes of 4 L and 2 L. *p<0.05, **p<0.01, ***p<0.001 taken as a significant differences.

	4 L		**2 L**	
Flow rates (L/min)	**% FPD (nominal)**	**MMAD (µm)**	**% FPD (nominal)**	**MMAD (µm)**
10 vs 20 L/min	***p<0.001	**p<0.01	***p<0.001	***p<0.001
10 vs 28.3 L/min	***p<0.001	***p<0.001	***p<0.001	***p<0.001
10 vs 40 L/min	***p<0.001	***p<0.001	***p<0.001	***p<0.001
10 vs 60 L/min	***p<0.001	***p<0.001	***p<0.001	***p<0.001
20 vs 28.3 L/min	***p<0.001	***p<0.001	***p<0.001	***p<0.001
20 vs 40 L/min	***p<0.001	***p<0.001	***p<0.001	***p<0.001
20 vs 60 L/min	***p<0.001	***p<0.001	***p<0.001	***p<0.001
28.3 vs 40 L/min	***p<0.001	***p<0.001	***p<0.001	***p<0.001

28.3 vs 60 L/min	**p<0.01	***p<0.001	**p<0.01	***p<0.001

40 vs 60 L/min	p = 0.381	***p<0.001	p = 0.381	***p<0.001

Table 5.64: Statistical comparison of the % mean nominal dose emitted from the Easyhaler® using inhalation volumes of 2 L and 4 L at flows of 10, 20, 28.3, 40, 60 L/min. *p<0.05, **p<0.01, ***p<0.001 taken as a significant differences.

	% Mean nominal fine particle dose
4 L vs 2 L 10 L/min	***p<0.001
4 L vs 2 L 20 L/min	***p<0.001
4 L vs 2 L 28.3 L/min	***p<0.001
4 L vs 2 L 40 L/min	***p<0.001
4 L vs 2 L 60 L/min	***p<0.001

Table 5.65: Statistical comparison of the % mean nominal fine particle dose and MMAD from the Turbuhaler® at varying flow rates of 10, 20, 28.3, 40, 60 L/min using inhalation volumes of 4 L and 2 L. *p<0.05, **p<0.01, ***p<0.001 taken as a significant differences.

	4 L		**2 L**	
Flow rates (L/min)	**% FPD (nominal)**	**MMAD (µm)**	**% FPD (nominal)**	**MMAD (µm)**
10 vs 20 L/min	***p<0.001	***p<0.001	***p<0.001	***p<0.001
10 vs 28.3 L/min	***p<0.001	***p<0.001	***p<0.001	***p<0.001
10 vs 40 L/min	***p<0.001	***p<0.001	***p<0.001	***p<0.001
10 vs 60 L/min	***p<0.001	***p<0.001	***p<0.001	***p<0.001
20 vs 28.3 L/min	***p<0.001	***p<0.001	**p<0.01	***p<0.001
20 vs 40 L/min	***p<0.001	***p<0.001	***p<0.001	***p<0.001
20 vs 60 L/min	***p<0.001	***p<0.001	***p<0.001	***p<0.001
28.3 vs 40 L/min	**p<0.01	***p<0.001	*p<0.05	***p<0.001

28.3 vs 60 L/min	***p<0.001	***p<0.001	**p<0.01	***p<0.001

40 vs 60 L/min	***p<0.001	***p<0.001	**p<0.01	***p<0.001

Table 5.66: Statistical comparison of the % mean nominal dose emitted from the Turbuhaler® using inhalation volumes of 2 L and 4 L at flows of 10, 20, 28.3, 40, 60 L/min. *p<0.05, **p<0.01, ***p<0.001 taken as a significant differences.

	% Mean nominal fine particle dose
4 L vs 2 L 10 L/min	***p<0.01
4 L vs 2 L 20 L/min	***p<0.001
4 L vs 2 L 28.3 L/min	***p<0.001
4 L vs 2 L 40 L/min	***p<0.001
4 L vs 2 L 60 L/min	***p<0.001

Table 5.67: Statistical comparison of % mean nominal fine particle dose from Aeroliser® after one inhalation and two inhalations. *p<0.05, **p<0.01, ***p<0.001 taken as a significant differences.

	4 L **% Mean nominal fine particle dose**	**2 L** **% Mean nominal fine particle dose**
One vs two inhalations **10 L/min**	***p<0.001	**p<0.01
One vs two inhalations **20 L/min**	***p<0.001	***p<0.001
One vs two inhalations **28.3in**	***p<0.001	***p<0.001
One vs two inhalations **60 L/min**	***p<0.001	***p<0.001
One vs two inhalations **90 L/min**	**p<0.01	***p<0.001

Table 5.68: Statistical comparison of % mean nominal fine particle dose from Easyhaler® after one inhalation and two inhalations. *p<0.05, **p<0.01, ***p<0.001 taken as a significant differences.

	4 L **% Mean nominal fine particle dose**	**2 L** **% Mean nominal fine particle dose**
One vs two inhalations 10 L/min	p = 0.361	p = 0.103
One vs two inhalations 20 L/min	p = 0.259	p = 0.362
One vs two inhalations 28.3in	p = 0.1286	p = 0.257
One vs two inhalations 40 L/min	*p<0.05	*p<0.05
One vs two inhalations 60 L/min	*p<0.05	*p<0.05

Table 5.69: Statistical comparison of % mean nominal fine particle dose from Turbuhaler® after one inhalation and two inhalations. *p<0.05, **p<0.01, ***p<0.001 taken as a significant differences.

	4 L **% Mean nominal dose fine particle**	**2 L** **% Mean nominal dose fine particle**
One vs two inhalations 10 L/min	p = 0.8399	*p<0.05
One vs two inhalations 20 L/min	p = 0.9825	*p<0.05
One vs two inhalations 28.3in	p = 0.0989	*p<0.05
One vs two inhalations 40 L/min	p=0.966	*p<0.05
One vs two inhalations 60 L/min	p = 0.196	*p<0.05

5.4 Discussion

The introduction of mixing inlet valve has enabled the determination particle size deposition pattern in the Anderson Cascade Impactor (ACI) at inhalation flows below 28.3 L/min. The use of the mixing inlet valve also prevents the inhalation flow within the ACI having an effect on the results and altering cut-off diameter (Nadarassan et al, 2009).

The results have shown that the formoterol particle size and its distribution from the Aeroliser, Easyhaler, and Turbuhaler are strongly dependent on the inhalation flow through the device. Also the total emitted dose revealed an increase in the total emitted dose (as % nominal dose) as inhalation flow rate increased (Figures 5.3-5.5 and Tables 5.1-5.20 and 5.61; Figure 5.7-5.9 and Tables 5.21-5.40 and 5.63; Figure 5.11-5.13 and Tables 5.41-5.60 and 5.65, respectively).

Several in vitro studies have demonstrated the inhalation flow rate dependency of DPI (Schultz et al, 1992, Hindle et al, 1994, Hindle et al, 1995). Several in vivo studies have also been reported on inhalation flow dependency of DPI. One of such studies carried out by Newman et al (1991) reported that when 10 asthmatic patients inhaled terbutaline from a Turbuhaler® at slow (28.3 L/min) and fast (60 L/min) rates, significantly more drug was deposited in the lung using a fast inhalation flow rate than with the slow rate (Newman et al, 1991).

Weuthen et al (2002) have compared the particle size distribution and MMAD for two formulations of formoterol (Oxis Turbuhaler® and Foradil Aeroliser®) at various inhalation flows of 28.3, 40, 60, and 80 L/min using ACI. The study showed an increase in fine particle dose emitted with increased inhalation flow rates (Weuthen et al, 2002). The determination of the fine particle distribution shows increase in the deposition of the fine particle mass with an increase in inhalation flow rate. This can be explained as a result of an effective de-aggregation of powder formulation in the inhaler when a high

inhalation flow is achieved. When comparing the performance of Turbuhaler, Easyhaler, and Aeroliser at different flow rates and inhalation volumes, it was observed that the fine particle mass for all three devices was significantly higher at inhalation volume of 4 L as compared to 2 L (Figures 5.3-5.5 and Tables 5.1-5.20 and 5.62; Figure 5.7-5.9 and Tables 5.21-5.40 and 5.64; Figure 5.11-5.13 and Tables 5.41-5.60 and 5.66, respectively). The data also demonstrated an increase in the fine particle dose of formoterol from Aeroliser® and Easyhaler® at lower inhalation flow rates using two inhalations compared to Turbuhaler® which required higher flow rates to disintegrate the powder into fine particle dose. The majority of DPIs contain formulation that is a mixture of lactose with micronised drug to improve flow. On the other hand, Turbuhalers use a different pharmaceutical principle to improve flow in their formulations. Particles are spheronised into small beads which require higher flow rate to disintegrate particles into fine particle dose. Such data is in agreement with studies by Weuthen et al (2002) who compared the fine particle size distribution between Turbuhaler® and Aeroliser® and reported increase in fine particle dose at high inhalation flow rate for Turbuhaler® and low flow rates for Foradil Aeroliser®. Such changes indicate that although all three DPI are dependent on inhalation flow rates to achieve a good fine particle dose other factors such as the intrinsic resistance of the DPI devices are also of great importance in determining the fine particle mass. The more resistance there is inside the inhaler then the lower will be the inhalation flow for a set inspiratory effort. Turbuhaler® is a high resistance device thus about the same amount of inspiratory flow rate produces a considerably lower flow through a device compared to one of low resistance (Clark and Hollingworth, 1993). On the other hand, Aeroliser® is low resistance DPI device hence the same amount of inspiratory flow will produce higher flow through the device. Interestingly, Easyhaler® is a device-metered DPI with a relatively high intrinsic

resistance. However, in-vitro tests have shown that the dose delivery from Easyhaler® is consistent and accurate starting from a minimum of 28.3 L/min through the inhaler (Vidgren et al, 1995). In line with this, the present data demonstrated that at a relatively low flow rate of 28.3 L/min the mean total emitted dose at one inhalation (SD) from Turbuhaler®, Aeroliser®, and Easyhaler® were 63.66% (1.57), 68.18% (0.76), 85.38% (3.91), respectively. Furthermore, at higher flow rates of 60 L/min the mean total emitted dose (SD) from Turbuhaler®, Aeroliser®, and Easyhaler® were 73.16% (2.62), 82.33% (0.60), 90.15% (3.39), respectively. This result is in agreement with studies by Chew and Chan (2001) and Weuthen et al (2002) which focused on two DPI devices with different intrinsic resistance (Foradil Aeroliser® and Oxis Turbuhaler®). Both studies demonstrated that Turbuhaler® delivers about less formoterol aerosol than Aeroliser®. Aeroliser® delivered more constant doses of approximately 80% of the total nominal dose at flow rates of 60 L/min or higher (Weuthen et al, 2002). The present results suggest that Aeroliser® and Easyhaler® are favourable for children or those suffering from lung disease. Conversely, the higher resistance of the Turbuhaler®, influences the ability of inhaling through it, thus making it more problematic, especially for asthma sufferers (Weuthen et al, 2002).

Furthermore, with regards to high intrinsic resistance inhalers, a previous study using Turbuhaler® showed that all COPD patients were able to generate peak inspiratory flow through the inhaler of ≤ 28 L/min (Dewar et al, 1999). However, in vitro tests suggest that optimal dosing with Turbuhaler® would require flows of at least 40–60 L/min due to Turbuhaler's high intrinsic resistance (Palander et al, 2000).

On the other hand, recent studies by Malmberg et al (2010) showed that almost all patients with COPD (91 of 93 patients) had an inspiratory effort sufficient to generate inspiratory flows through the Easyhaler®, regardless of the relative high resistance of the inhaler.

Although DPIs with low internal resistance are more suitable for patients with more severe airway obstruction, the turbulent energy is a product of the flow and the inhaler's resistance, for a set energy level needed for de-aggregation of the drug particles, the flow required through a low resistance DPI will be higher than that of a high-resistance DPI (Chrystyn, 2003). With a high-resistance DPI, the inspiratory flow through the inhaler will be determined mostly by the inhaler's resistance and is less affected by varying airway resistance of the patient, which may result a more consistent dose emission (Clark, 1994). The Easyhaler® is good example of an inhaler with a relatively high internal resistance and consistent dose delivery over a wide range of inhalation flow rate (Palender, 2000). This correlates with the present data where lower inhalation flow rates of 20 and 28.3 L/min were able to achieve sufficient inspiratory effort through Easyhaler® and good drug delivery (71.63% and 85.38%, respectively).

This data agrees with a previous study of young children with asthma who also showed sufficient inspiratory flows through Easyhaler® (Malmtrom et al, 1999). Furthermore, a study with pediatric and adult asthmatic patients has confirmed that clinical efficacy of salbutamol is maintained even at low inspiratory flow rates when using Easyhaler® (Koskela et al, 2000).

The results of MMAD showed a significant ($p<0.01$-$p<0.001$) decrease in particle size for all three DPI devices as inhalation flow rates increased. The MMAD (SD) produced from Turbuhaler® at flow rates of 10, 20, 28.3, 40 and 60 L/min were 6.52 (0.16), 4.56 (0.05), 3.84 (0.05), 3.30 (0.07), and 2.78 μm (0.08), respectively. Similarly, the MMAD (SD) produced from Easyhaler® were 5.61 (0.65), 4.50 (0.10), 3.76 (0.05), 3.28 (0.15), 2.76 (0.09), μm respectively. The MMAD (SD) produced from Aeroliser® at flow rates of 10, 20, 28.3, 60, and 90 L/min were 6.22 (0.16), 5.74 (0.09), 4.44 (0.09), 3.20 (0.07), 2.90 μm (0.00),

respectively.

This decrease in particle size with increased flow rate may be due to the release of particle from their carrier particles at a higher flow (Clark and Hollingworth, 1993). It has also been suggested that the MMAD is dependent on the peak inspiratory flow. Hence, a decrease in MMAD values suggests that the inhaler device produces finer aerosol particles, which are more uniformly distributed and therefore increase the delivery of the drug to the peripheral airways of the lungs.

In line with this, several factors including inspiratory flow and the varying intrinsic resistance of DPI devices may limit the ability of young children and those with severe obstructive disease to generate sufficient inhalation flow through DPI devices. This creates the risk of reduced bronchodilation response during episodes of acute wheezing or in patients with low lung function (Pendersen, 1987). Also, for using single dose DPI, the manufacturers tend to recommend inhaling twice and as deep as possible from each dose in order to maximise the total dose emitted. On the other hand, the manufacturer drug information leaflet for using available multi-dose DPIs recommends only one inhalation per dose and patients should inhale as fast as they can. Abdelrahim et al (2009) analysed in-vivo the effect of inhaling twice using Turbuhaler® (multi-dose DPI). As it has a very high resistance, patient inhalation flow through the device would be low. The total emitted dose of 500 μg terbutaline sulphate from a Turbuhaler® was determined using inhalation flow rates of 10 to 60 L/min with inhalation volume of 2 and 4 L. The relative lung and systemic bioavailability of terbutaline from Turbuhaler® was then determined in healthy subjects and COPD patients after one and two inhalations at slow and fast inhalation flows using a urinary terbutaline pharmacokinetic method. Results showed that the nominal dose from the one and two inhalations increased significantly ($p < 0.05$) with the increase of the inhalation flow at both 2 and 4 L inhalation volumes. Additionally, the relative lung and systemic bioavailability after

one inhalation at fast inhalation flow were significantly higher ($p < 0.01$) than at slow inhalation flow in both healthy subjects and patients. Also, the author found that there is a significant increase in the lung deposition at 4 L compared to 2L. Moreover, the author reported that there was no significant difference between one and two inhalations in the amount excreted in urine for healthy subject which attributed to large inhalation volume (4 L) in healthy subjects, which was the opposite case in patients with small volume. Therefore, the author suggested that for a patient who has small inhalation volume it would be better to inhale twice from the Turbuhaler® (Abdelrahim et al, 2009). Results in the present study are in agreement with those reported by Abdelrahim et al (2009). In the present study in-vitro method was used to determine total emitted dose of formoterol from Aeroliser®, Turbuhaler®, and Easyhaler® at inhalation flow rates of 10-90 L/min using inhalation volumes of 4 L and 2 L after one and two inhalation. The present data revealed that there were no significant differences in both nominal dose and fine particle dose of formoterol from Turbuhaler® following one and two inhalations at flow rates of 10-60 L/min using 4 L inhalation volume. At inhalation volume of 2 L the fine particle dose from one and two inhalations was significantly ($p<0.05$) higher at inhalation flow rates of 10-60 L/min (Figures 5.11-5.13 and Tables 5.41-5.60 and 5.69).

The nominal dose and fine particle dose of formoterol from Easyhaler® following two inhalations was significantly higher ($p<0.05$) at higher flow rates of 40 and 60 L/min compared to one inhalation, but there was no significant difference at lower flow rates of 10, 20, and 28.3 L/min.
The nominal dose and fine particle dose of formoterol from Aeroliser following one and two inhalation increased significantly ($p<0.01$-$p<0.001$) with increase in inspiratory flow rate at both 4 L and 2 L inhalation volumes. These finding are in accordance with those reported by Al-Fadhl et al (2007) who reported that the total dose emission and

fine particle dose emitted from a tiotropium Handihaler® (single dose device) increased with increase of flow rate (10-50 L/min) and also the fine particle dose for two inhalation were higher that one inhalation. So this research work indicates the relevance of inhaling twice and as deep and hard as possible from each dose from DPI devices. Especially, for patients with low inspiratory flow and limited inhalation volume as they may not receive much benefit from one inhalation.

In summary, all three DPI devices (Turbuhaler®, Easyhaler®, and Aeroliser®) are highly dependent on inhalation flow rate, producing higher amounts of fine particle dose with increased inhalation flow rate. Moreover, due to design differences in DPI devices, fine particle dose delivered from DPI devices was shown to be dependent not only on inhalation flow rates following a specific inhalation effort but the specific intrinsic resistance of each DPI device. As a result low resistance DPI devices such as Aeroliser® delivered higher fine particle dose compared to high resistance Turbuhaler® with the same inhalation effort. Interestingly, Easyhaler®, a relatively high intrinsic resistance DPI device delivered the highest fine particle dose of formoterol with the same inhalation effort. The MMAD was also highly dependent on inhalation flow rate with higher flow rates producing finer particle size and increased lung deposition. The present data also showed that two inhalations from a single dose increased the fine particle dose at varying flow rates and inhalation volumes.

5.5 Conclusion

The choice of the inhaler device is an important part of the management of patients with asthma and COPD.

The present data suggest that although fast inspiratory flow is required in order to produce high fine particle dose, the use suggests that young children and those with

severe obstructive disease are more likely to have problems using a fast inhalation flow. However, low resistance devices such as Aeroliser® seems to be highly favourable as they deliver higher fine particle dose at lower flow rates. Additionally, the use of two inhalations from each dose seems to be highly favourable, especially from Aeroliser®. Conversely, Turbuhaler® is less favourable as it requires fast inhalation flow in order to deliver optimal fine particle dose. Interestingly, Easyhaler® also seems to be highly favourable as it showed to deliver high fine particle dose at relatively low inhalation flow rates and additionally at higher flow rates the use of two inhalations significantly increases fine particle dose delivery. This is indeed desirable, especially for young children, COPD and asthma sufferers, elderly, and those that have poor lung function who may not be able to generate fast inhalation flows.

CHAPTER SIX

IN-VITRO EVALUATION OF THE DOSE EMISSION CHARACTRISTICS OF FORMOTEROL FROM ATIMOS MODULITE® MDI

6.1 Introduction

The efficacy of inhalation therapy is dependent in a variety of parameters determining the drug deposition within the lungs. These include the output of the inhalation device, which may be dependent on the inhalation flow rate and inhalation flow profile. The particle size distribution of the emitted aerosol, which may also be dependent on the inhalation flow rate and inhalation flow profile. Additionally, lung disease may equally change the deposition characteristics of particles in the lungs.

The efficacy of such devices should ideally be determined in in-vivo studies in human volunteers under relevant conditions. However, as such approaches are expensive and time-consuming an alternative approach would be to use in vitro techniques for device characterisation that estimates human lung deposition. Such approach addresses the relationship between device output, particle size distribution and inhalation flow rates. Additionally, in vitro characterisation of an aerosol is useful as a pre-clinical tool to predict the amount of fine particles available upon inhalation.

Modulite® technology is an aerosol system based on propellant hydrofluoroalkanes (HFA). The original aim of the Modulite® technology is to provide CFC-free formulations and match CFC-based MDI in terms of aerosol characteristics. Modulite® technology allows the size and distribution of particles to be tailored, enabling the drug delivery to be targeted to different parts of the lung.

MDI is a low emission-resistance device and so it is easier to generate a high inspiratory flow rate through it. As a result many patients inhale too fast (>90 L/min) through a MDI, resulting in increased oropharyngeal deposition and less lung deposition, which provides less therapeutic benefit from the medication (Al-Showair et al, 2007).

Although the Pharmacopeias recommend flow rate of 28.3 L/min for particle size distribution, the in vivo studies have shown that a slow inhalation rate of 20 L/min with

a MDI can increase the relative bioavailability of salbutamol to the lung compared with faster inhalation rate of 60 L/min (Tomlinsonet al, 2005).

Moreover, the British Pharmacopeia recommends an inhalation volume of 4 L across the device, but patients with severe lung disorders cannot achieve the 4 L. Thus it is necessary to understand the effects of aerodynamic particle profile when different inhalation volumes are used.

The present study was designed to determine the formoterol fumarate dose emitted from an Atimos® Modulite® using peak inhalation rates of 10, 28.3, 60, and 90 L/min that correspond to normal patient use and with inhalation volumes of 4L and 2L. The study was also carried out to determine the uniformity of dose emitted from the Atimos Modulite® and to identify inter and intra inhaler dose emission variability from six different batches of Atimos Modulite® device.

Moreover, the aerodynamic properties of dose emitted from an Atimos Modulite with and without spacer at flows of 15, 28.3, 50, 60 L/min using mixing inlet at inhalation volume of 4L was also determined

6.2 Methods and Instrumentation

6.2.1 Equipment and inhalation device

Equipment were used as described in chapter 3 (section 3.1).

HPLC analysis was carried out as explained in section (3.1.1 and 3.2.1)

The various equipment used for the Anderson Cascade Impactor study are explained in Chapter 3 (sections 3.1.2 and 3.1.3).

6.2.2 Instrumentation set-up

The dose emission method described in the compendial method (USP, 2007) for MDI was used. The sampling apparatus for MDI has a smaller internal diameter to accommodate 25 mm disks compared to the one used for DPI. This feature enables collection of dose at high inhalation flows, up to 100L/min (USP, 2007). The mouth piece ensures that there is no sample loss between the collection tube and the inhaler mouth piece. A vacuum pump with excess capacity was selected in order to achieve a desired inhalation flow. A timer controlled two-way solenoid valve (P2 and P3) was connected between the vacuum pump and the flow controller. This valve enabled a set volume of air to be withdrawn from the mouth piece of the inhaler at a designated inhalation flow (USP, 2007). Flow control was achieved ensuring that critical (sonic) flow occurs in the flow control valve (absolute pressure ratio $P3/P2 \leq 0.5$ under steady flow conditions).

The inhalation time was set using the relation ($T = (60\text{sec} \times X)/Q$, where T represents the time consistent for withdrawal of 4L or 2 L of air via the inhaler; Q, the airflow; and X represents the 2 or 4L to be drawn through the inhaler.

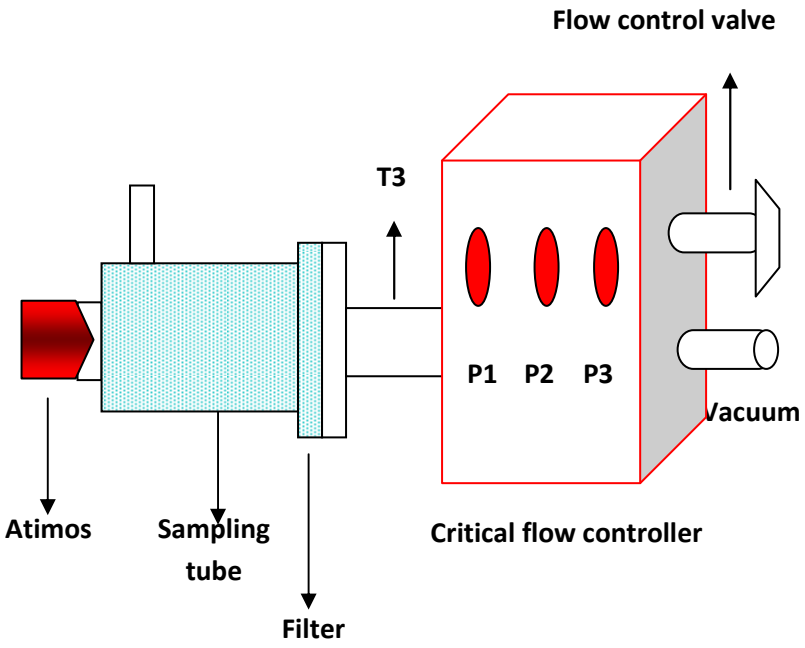

Figure 6.1: Schematic illustration of instrumentation for dose emission measurement from an Atimos® Modulite®.

6.2.3 Dose emission measurement

The total emitted dose of formoterol fumarate from an Atimos® Modulite® (Trinity Chiese, Italy) containing 12 µg of formoterol fumarate per dose was determined by using sampling apparatus USP (2007) at inhalation flows of 10, 28.3, 60, 90 L/min. The, instrument was assembled as per the schematic diagram in Figure 6.1. A glass fibre filter, 25 mm (Pall Gelman Sciences, USA) was used to collect the drug in the sampling apparatus. The vacuum pump was switched on 20 minutes prior to each dose emission experiment in order to attain a stable flow. The two way solenoid valve was opened and the desired inhalation flow (10, 28.3, 60, 90 L/min) was adjusted using the flow control valve. Once the flow was set, the Atimos® Modulite® inhaler device was inserted into the mouthpiece horizontally, with the vacuum pump still running and the solenoid valve closed. Parafilm was used in order to seal the mouthpiece to prevent any drug loss during the experimental procedure. The dosing time was calculated for each inhalation

flow using the equation explained in section 6.2.2. The MDI was actuated and the dose was discharged into a sampling apparatus by activating the timer controlling the solenoid valve (USP, 2007, EP, 2007) so that the set inhalation volume of air was passed through the inhaler. The change in pressure (P1) across the inhaler was also monitored simultaneously and recorded at each inhalation flow (10, 28.3, 60, 90 L/min). Similarly, ten separate single doses were determined at each inhalation flow and each inhalation volume of 2L and 4L.

Randomised sampling schemes were used for studying the dose uniformity over the entire 100 actuations of Atimos® Modulite®. Doses from the inhaler which were not used were discharged to waste using an inhalation flow of 60 L/min.

After the dose was discharged into the apparatus the inhaler device was detached. The collection filter was carefully removed from the sampling tube and was completely immersed in an airtight sample bottle (to prevent evaporation of methanol) containing 20 mL water of 80% methanol. The sample bottle was placed in a sonic bath for 5 minutes. This procedure was repeated two times (to ensure the entire drug leads in the filter was released). Preliminary study was performed to validate the amount of drug left over in the filter and it was confirmed that the entire drug leads in the filter was effectively removed during the second wash.

The sampling tube was closed from one end and a 20 mL water of 80% methanol was filled in the sampling tube. The other end was sealed using parafilm. The tube was shaken so as to dissolve any remaining drug particle. All the solutions were then mixed together and filtered using a membrane filter. The resultant solution was transferred into volumetric flasks and diluted to appropriate volumes prior to quantitative high performance liquid chromatography (HPLC) analysis. The HPLC method is described in chapter 3 (sections 3.1.1 and 3.2.1).

6.2.4 Procedure to set up Anderson Cascade Impactor with the mixing inlet valve

The parts of the Anderson Cascade Impactor (ACI) and respective accessories induction port were washed in methanol and dried at room temperature. The collection plates were then sprayed with silicone and then allowed to dry 1 hour to analysis. After the drying process the ACI stages were assembled with a GF 50 (Copley Scientific Ltd, UK) filter located in the final stage. The inhalation time was set and the mixing inlet valve was connected between the USP induction port and ACI. A schematic design is shown in Figure 6.2.

6.2.4.1 Setting up the ACI for low flow rates: 15 and 28.3L/min

The mixing inlet was connected to the top stage (stage zero) of the impactor and then the induction port was connected to the top of the mixing inlet (Figure 6.2.). The ACI was set to be at 28.3 L/min. The mixing inlet valve consists of an outer and central channel. The inhaler aerosol travelled through the central channel and the air supply from compressed air travelled along the outer channel.The mixing inlet valve side arm was closed by using parafilm. The MDI was attached to the spacer. The ACI apparatus was then connected to the critical flow controller (model TKP). The flow control valve was adjusted until the flow, measured by the flow meter (MKS) attached to the chamber was equal to 28.3 L/min (sonic flow was achieved $P3/P2 \leq 0.5$). The inhalation flow was switched to bypass the flow controller. The side arm of the mixing inlet valve was opened and a source of a compressed air was connected to it. Compressed air was introduced through the side arm of the mixing inlet valve to compensate for the required flow of 28.3L/min through the ACI. For example to achieve a desired flow of 15 L/min through the inhaler device, a 13.3 L/min flow of compressed air was introduced through the side port of the mixing outlet valve.

6.2.4.2 Setting up the ACI for high flow rates: 50 and 60L/min

The ACI was set to be at 60 L/min so stage 0 and 7 were replaced by -1 and -0. Thus cut off diameter would not be altered by using high flow rate. The mixing inlet valve side arm was closed by using parafilm. The air flow through the cascade impactor was adjusted and remained constant at a set value of 60 L/min (sonic flow was achieved P3/P2≤0.5) throughout the experiment. The side arm of the mixing inlet valve was opened and a source of a compressed air was connected to it. The addition of the compressed air allowed the desired test flow rates to be achieved, while keeping the necessary 60L/min air flow through the ACI.

The mixing inlet valve was still attached for the other two flow rates of 28.3L/min and 60L/min, however the outer valve of the mixing inlet was then closed off using parafilm as the addition of compressed air for these flow rates was deemed unnecessary given the fact that the ACI has been designed for use at flow rates of 28.3L/min and above e.g. 60L/min.

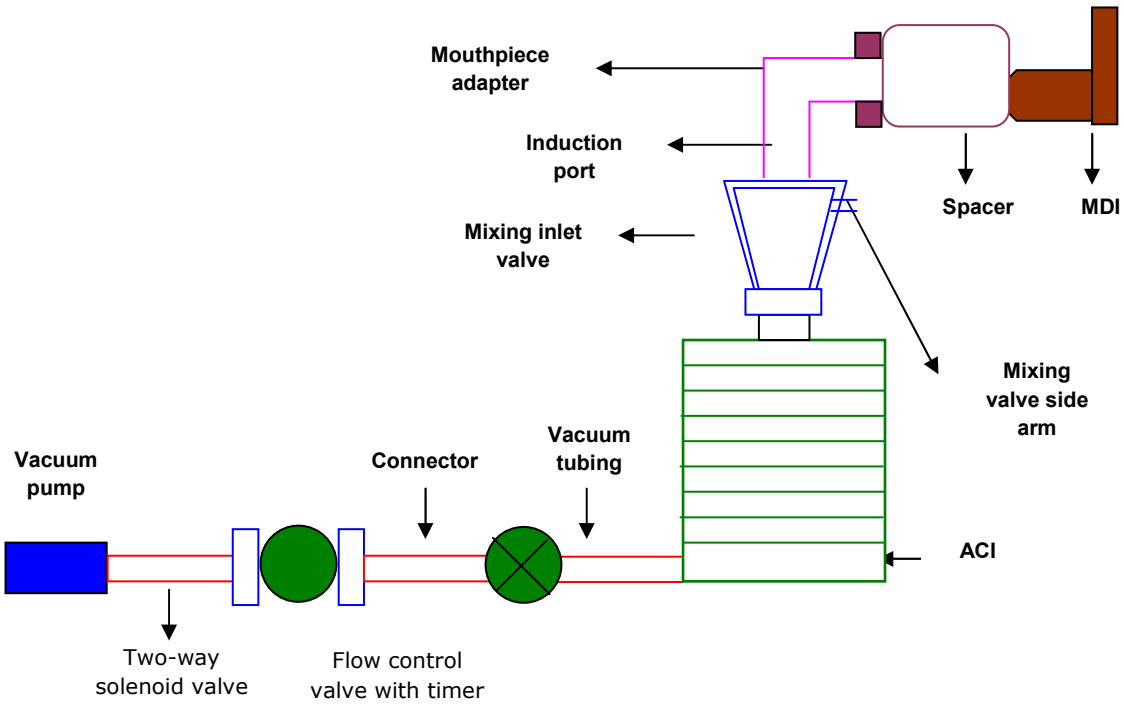

Figure 6.2: Anderson Cascade Impactor with mixing inlet valve.

6.2.5 High performance liquid chromatography (HPLC) analysis

The amount of drug deposited on stages of the impactor was analysed using HPLC as explained in Chapter 3 (section 3.3.1 and section 3.3.2 and section 3.3.3).

6.2.6 Fine Particle analysis

The Copley software (CITDAS version 2.0) was used to calculate the aerodynamic dose emission parameters. The fine particle size dose was calculated using the total mass of (R), of the drug deposited on the stages and filter of the cascade impactor that captures the drug in the fine particle size range.

Fine particle dose is equal to R/n, where the fine particle dose refers to the amount < 5 µm and n refers to the number of doses discharged.

Fine particle fraction is a ratio R (as described above) with the total mass of drug delivered from the mouthpiece of the inhaler into the apparatus.

Fine particle fraction is equal to $R/\sum A$.

The mass median aerodynamic diameter (MMAD) was obtained from the logarithm of the effective cut-off diameter corresponding to 50% undersize (on a log probability scale). The geometric standard deviation (GSD) was the square root for the size corresponding to 84.13% less than the stated size divided by the square root of the size for 15.87%.

6.2.7 Statistical Analysis

Analysis of variance was performed to determine the effect of inhalation flow and to compare doses emitted by each inhaler at the different inhalation flow using inhalation volume of 2L and 4L. Moreover, the effect of varying inhalation flows on fine particle distribution without using spacer and with Aerochamber Plus and Volumatic spacers were also studied. This was carried out using one way ANOVA with the application of Bonferoni's correction to determine the difference between paired data.

6.3 Results

6.3.1 Dose emission at varying inhalation flows of 10, 28.3, 60, and 90 L/min using 4 L and 2 L inhalation volumes

The in-vitro dose emission performance of formoterol total Atimos® Modulite® was studied at varying inhalation flow rates of 10, 28.3, 60, and 90 L/min using volumes of 4 and 2 L. The total dose emission for 10 separate inhalations from each of six inhalers was determined.

The results show a consistency in the total emitted dose as well as the percentage of emitted dose of formoterol across a range of 10-90 L/min at both 4 L (Table 4.1 and Figure 4.3) and 2 L (Table 4.2 and Figure 4.4) inhalation volumes. Moreover, the emitted dose obtained from a 2 L inhalation volume is nearly the same as of the amount obtained using 4 L.

Table 6.1: Total emitted dose of formoterol fumarate (%nominal dose) from Atimos® Modulite at varying inhalation rates of 10, 28.3, 60, and 90 L/min using inhalation volumes of 4 L.

Dose	Flow rate (L/min)							
	10		28.3		60		90	
	Amount (µg)	Emitted %	Amount (µg)	Emitted %	Amount (µg)	Emitted %	Amount (µg)	Emitted %
1	11.87	98.96	11.51	95.89	12.03	100.22	11.89	99.09
2	13.29	110.73	11.91	99.24	13.57	113.06	11.31	94.25
3	11.48	95.69	12.61	105.08	11.33	94.43	12.91	107.61
4	13.48	112.36	12.19	101.60	11.94	99.46	13.96	116.34
5	14.14	117.80	12.13	101.09	11.67	97.22	12.14	101.18
6	10.89	90.73	11.20	93.36	11.97	99.71	11.02	91.85
7	10.43	86.95	11.78	98.20	9.87	82.23	12.25	102.11
8	13.18	109.85	10.65	88.79	11.73	97.72	12.29	102.39
9	11.60	96.66	11.55	96.25	13.73	114.43	13.52	112.69
10	12.57	104.77	13.37	111.38	12.00	99.96	11.10	92.51
Mean	12.29	102.45	11.89	99.09	11.98	99.84	12.24	102.00
S.D.	1.22	10.17	0.75	6.28	1.09	9.06	0.99	8.26

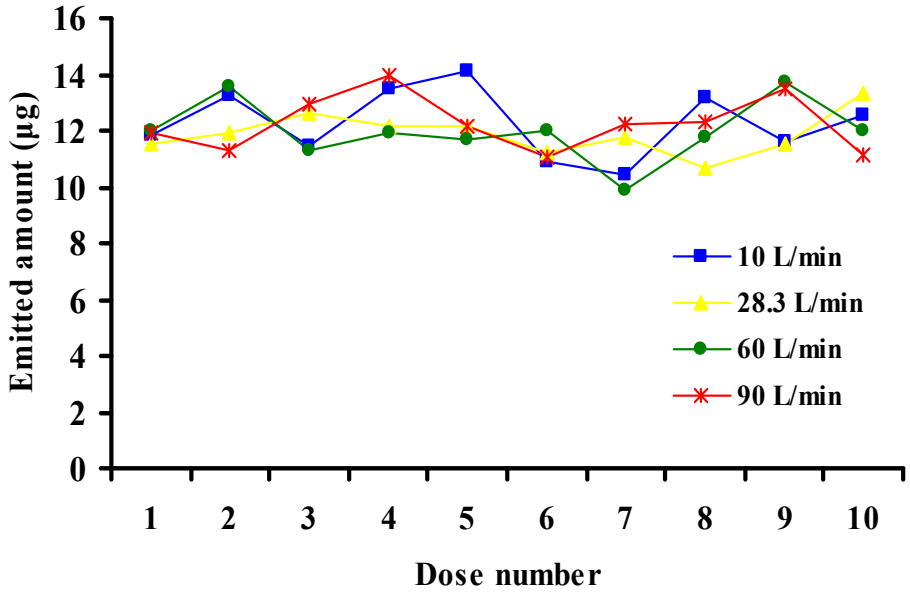

Figure 6.3: Mean total emitted dose of formoterol (µg) from Atimos® Modullite® following varying inhalation rates of 10, 28.3, 60, 90 L/min using inhalation volume of 4 L.

Table 6.2: Total emitted dose of formoterol fumarate (%nominal dose) Atimos® Modulite at varying inhalation rates of 10, 28.3, 60, and 90 L/min using inhalation volumes of 2 L.

Dose	Flow rate (L/min)							
	10		28.3		60		90	
	Amount (µg)	Emitted %	Amount (µg)	Emitted %	Amount (µg)	Emitted %	Amount (µg)	Emitted %
1	11.60	96.67	11.93	99.39	11.91	99.23	8.22	68.48
2	13.66	113.81	12.09	100.71	13.25	110.44	8.77	73.06
3	12.59	104.90	11.28	93.96	11.20	93.30	12.75	106.24
4	10.05	83.73	10.84	90.35	13.31	110.90	10.81	90.08
5	12.41	103.46	10.78	89.81	12.60	105.04	14.24	118.70
6	12.75	106.26	10.99	91.61	12.29	102.41	12.65	105.40
7	12.73	106.12	13.03	108.59	10.81	90.06	10.93	91.11
8	12.23	101.91	11.10	92.48	12.08	100.69	13.22	110.13
9	12.51	104.24	13.55	112.92	12.21	101.75	13.42	111.85
10	13.09	109.11	12.97	108.12	12.60	104.98	10.39	76.59
Mean	12.36	103.02	11.86	98.80	12.23	101.88	11.54	96.16
S.D.	0.97	8.11	1.02	8.52	0.80	6.64	2.04	16.97

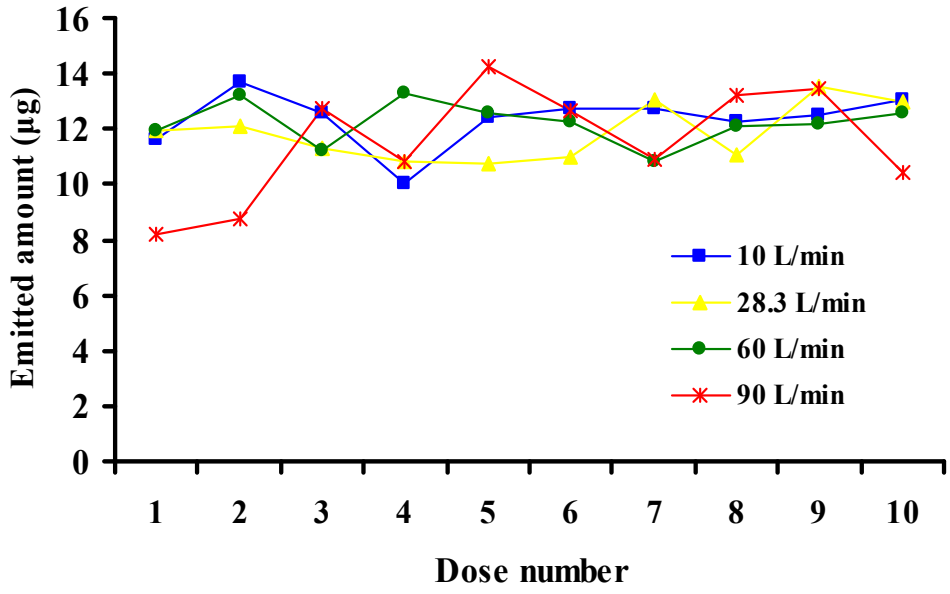

Figure 6.4: Mean total emitted dose of formoterol (µg) from Atimos® Modullite® following varying inhalation rates of 10, 28.3, 60, 90 L/min using inhalation volume of 2 L.

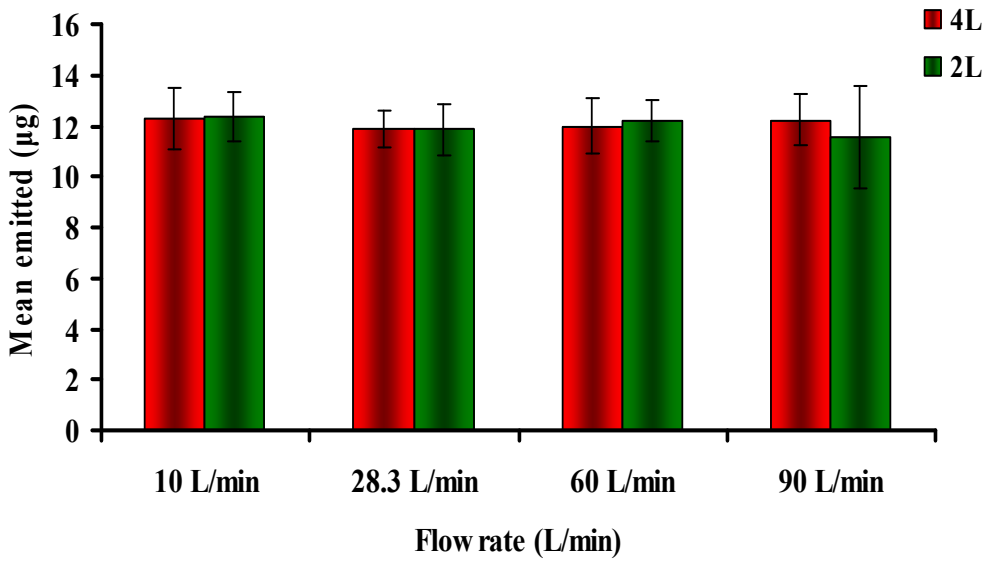

Figure 6.5: Mean total emitted dose of formoterol (µg) from Atimos® Modulite® following varying inhalation rates of 10, 28.3, 60, and 90 L/min using volumes of 4 and 2 L.

6.3.2 Particle size distribution from an Atimos® without and with Aerochamber Plus and Volumatic Spacers

The particle size distribution and fine particle dose (FPD), expressed as % nominal for formoterol at varying flows of 15, 28.3, 50 and 60 L/min with and without use of spacers are shown in Table 6.3-6.14.

The abbreviations used in the tables include total emitted dose (TED), fine particle dose < 5 μm (FPD), fine particle fraction (FPF), mass median aerodynamic diameter (MMAD), and geometric standard deviation (GSD).

The mean percentage nominal fine particle dose (SD) of formoterol emitted from an Atimos Modulite® at flows of 15, 28.3, 50 and 60 L/min were 62.40% (0.28), 63.41% (0.52), 64.71% (0.61), 58.43% (0.73), with Volumatic (Figure 6.11) and 63.62% (0.44), 63.86% (0.72), 64.72% (0.47), 59.96% (1.97) with Aerochamber (Figure 6.9) respectively. On the other hand, the mean nominal fine particle dose (SD) at flows of 15, 28.3, 50 and 60 L/min without spacers (Figure 6.7) were 53.52% (2.51), 54.1% (0.79), 53.37% (0.81), 50.43% (1.92) respectively. Results showed an increase in the fine particle dose emitted through a MDI with and without spacers at lower flow rates compared to higher flow rates (Figures 6.7, 6.9, 6.11, 6.13 and 6.14).

The performance of the Aerochamber Plus and volumatic in delivering formoterol Atimos® MDI was compared with results obtained from using the MDI alone. Results showed that MMAD values observed in the performance of the Aerochamber and Volumatic were smaller than using the MDI alone (Figure 6.15) as would be expected. When using the Aerochamber MMAD values were seen to increase as the flow rate was increased from 15 to 60 L/min, highlighting that at higher flow rates there is lower particle deposition (Figure 6.10). A similar increase was seen when Volumatic and when no spacers were used for the experiments (Figures 6.8 and 6.12).

Table 6.3: Aerodynamic particle profile at flows of 15 L/min without spacer.

Amount deposited on each stage (µg)						
Stage	**Run 1**	**Run 2**	**Run 3**	**Run 4**	**Run 5**	**Mean µg**
Induction port	20.38	15.35	14.35	12.34	30.42	18.57
0	0.77	0.81	0.85	0.97	0.77	0.83
1	0.72	0.74	0.72	0.82	0.72	0.74
2	0.82	0.78	0.82	0.78	0.82	0.80
3	19.84	19.78	20.85	16.92	18.97	23.87
4	22.00	22.02	24.03	22.16	22.14	22.47
5	12.86	12.90	12.96	13.95	11.98	12.93
6	6.77	6.88	8.07	8.03	4.88	6.92
7	2.00	2.00	1.40	2.15	1.54	1.82
Filter	0.00	0.00	0.00	0.00	0.00	0.00
TED (µg)	90.15	85.25	88.04	85.11	96.23	88.96
FPD (%nominal dose)	53.58	53.63	56.78	53.32	50.28	53.52
MMAD (µm)	3.47	3.12	3.45	3.33	3.45	3.36
GSD	2.17	2.31	2.19	2.15	2.19	2.20

Table 6.4: Aerodynamic particle profile at flows of 28.3 L/min without spacer.

Amount deposited on each stage (µg)						
Stage	**Run 1**	**Run 2**	**Run 3**	**Run 4**	**Run 5**	**Mean µg**
Induction port	11.30	11.30	11.30	11.30	11.30	11.30
0	3.38	3.96	3.51	4.27	3.85	3.79
1	2.05	2.63	2.19	2.94	2.52	2.47
2	3.20	3.79	3.34	4.10	3.67	3.62
3	12.59	13.07	11.19	11.33	13.26	12.29
4	15.30	15.31	15.20	15.00	15.38	15.24
5	18.18	18.10	18.20	17.9	17.95	18.07
6	11.58	11.55	11.56	11.51	11.50	11.54
7	3.41	4.02	4.55	4.69	4.21	4.18
Filter	0.00	0.00	0.00	0.00	0.00	0.00
TED (µg)	87.07	89.92	87.22	89.78	89.96	88.79
FPD (%nominal dose)	53.55	54.86	53.37	53.78	54.96	54.1
MMAD (µm)	3.49	3.49	3.45	3.54	3.65	3.52
GSD	2.11	2.19	2.11	2.16	2.19	2.15

Table 6.5: Aerodynamic particle profile at flows of 50 L/min without spacer.

Amount deposited on each stage (µg)						
Stage	**Run 1**	**Run 2**	**Run 3**	**Run 4**	**Run 5**	**Mean µg**
Induction port	18.72	18.72	18.72	18.72	18.72	18.72
-1	5.71	5.95	5.80	6.10	5.92	5.89
0	2.69	3.33	3.29	3.47	3.41	3.24
1	4.67	4.85	4.75	5.00	4.87	4.83
2	11.56	11.56	11.56	11.56	11.56	11.56
3	15.38	16.48	16.03	16.17	16.67	16.15
4	16.15	16.15	16.15	16.15	16.15	16.15
5	10.58	10.58	10.58	10.58	10.58	10.58
6	3.41	4.02	4.55	4.69	4.21	4.18
Filter	0.00	0.00	0.00	0.00	0.00	0.00
TED (µg)	88.87	91.64	91.42	92.44	92.10	91.29
FPD (%nominal dose)	51.46	53.04	53.01	53.46	53.37	53.37
MMAD (µm)	4.07	4.17	4.06	4.05	4.08	4.09
GSD	2.21	2.11	2.21	2.34	2.31	2.24

Table 6.6: Aerodynamic particle profile at flows of 60 L/min without spacer.

Amount deposited on each stage (µg)						
Stage	**Run 1**	**Run 2**	**Run 3**	**Run 4**	**Run 5**	**Mean µg**
Induction port	26.74	26.74	26.74	26.74	26.74	26.74
-1	2.06	3.43	2.27	2.41	3.62	2.76
0	18.35	19.09	15.64	15.71	19.19	17.60
1	3.15	1.85	3.21	3.28	1.94	2.69
2	8.31	8.04	7.98	8.05	8.14	8.10
3	12.83	12.56	12.88	12.95	12.66	12.77
4	17.74	17.74	17.74	17.74	17.74	17.74
5	14.15	11.65	7.08	7.15	11.74	10.35
6	3.65	7.03	7.04	7.11	7.15	6.40
Filter	5.14	5.14	5.14	5.14	5.14	5.14
TED (µg)	98.69	99.58	92.73	93.32	100.38	96.94
FPD (%nominal dose)	51.51	51.80	48.21	48.46	52.16	50.43
MMAD (µm)	4.12	4.15	4.17	4.17	4.19	4.16
GSD	2.13	2.41	2.36	2.14	2.16	2.24

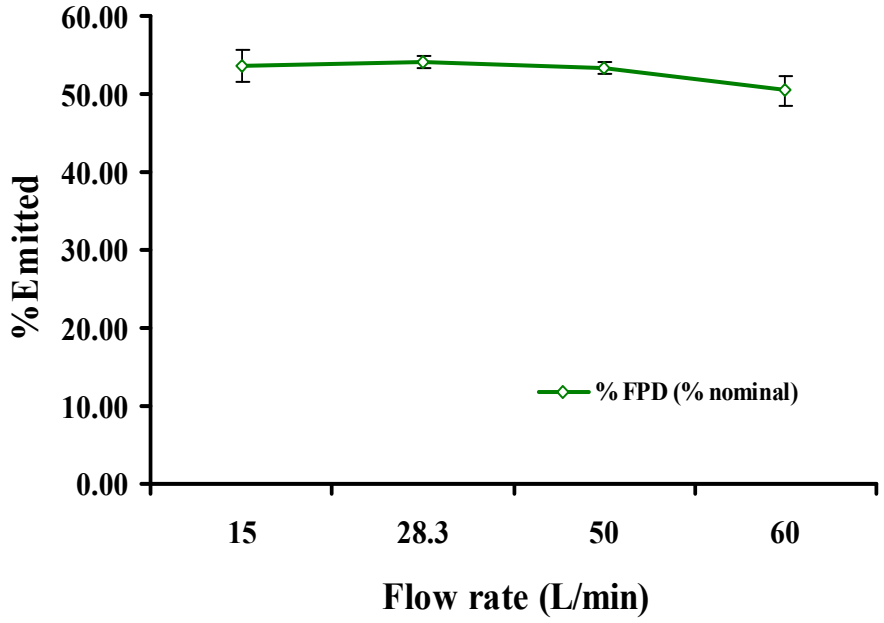

Figure 6.6: Comparison of %FPD (%nominal) at flow rate of 15, 28.3, 50 and 60 L/min without spacer.

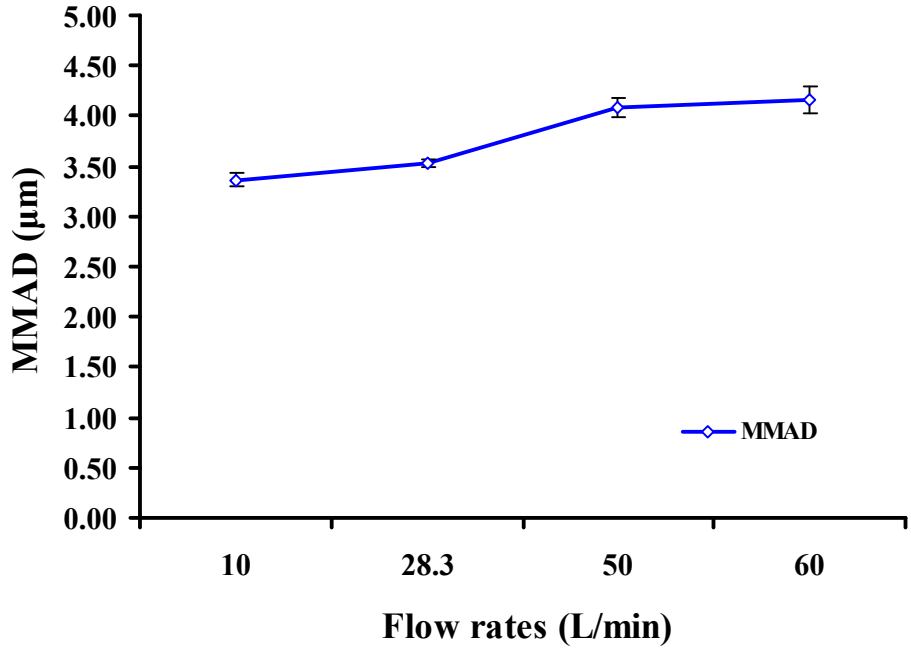

Figure 6.8: Comparison of MMAD (µm) at flow rate of 15, 28.3, 50, and 60 L/min without a spacer.

Table 6.7: Aerodynamic particle profile at flows of 15 L/min with an Aerochamber® plus spacer.

Amount deposited on each stage (µg)						
Stage	**Run 1**	**Run 2**	**Run 3**	**Run 4**	**Run 5**	**Mean (µg)**
Induction port	10.33	10.23	10.08	10.11	10.22	10.22
0	5.79	5.69	5.54	5.57	5.68	5.67
1	4.84	4.74	4.59	4.61	4.73	4.72
2	9.86	10.06	9.92	9.94	10.05	9.95
3	13.80	14.00	13.85	13.88	13.99	13.88
4	20.00	20.20	20.05	20.08	20.19	20.08
5	17.75	17.95	17.81	17.83	17.94	17.84
6	9.73	9.93	9.78	9.80	9.92	9.81
7	4.70	4.90	4.76	4.78	4.89	4.79
Filter	0.00	0.00	0.00	0.01	0.00	0.00
Delivered dose (µg)	96.79	97.69	96.38	96.60	97.61	96.95
Amount in spacer (µg)	9.68	9.77	9.64	9.66	9.76	9.70
TED (µg)	106.47	107.46	106.02	106.26	107.37	106.65

FPD (%nominal dose)	63.19	64.20	63.47	63.59	64.15	63.62
MMAD (µm)	2.79	2.79	2.83	2.81	2.80	2.80
GSD	2.03	2.09	2.10	2.10	2.05	2.07

Table 6.8: Aerodynamic particle profile at flows of 28.3 L/min with an Aerochamber® plus spacer.

Amount deposited on each stage (µg)						
Stage	**Run 1**	**Run 2**	**Run 3**	**Run 4**	**Run 5**	**Mean (µg)**
Induction port	9.31	9.44	9.06	9.16	9.31	9.27
0	5.79	5.92	5.55	5.65	5.79	5.75
1	4.94	5.07	4.70	4.80	4.94	4.90
2	10.05	10.17	9.80	9.90	10.04	10.00
3	14.21	14.34	13.97	14.07	14.21	14.17
4	20.20	20.32	19.95	20.05	20.19	20.16
5	17.95	18.08	17.71	17.81	17.95	17.91
6	9.75	9.87	9.50	9.60	9.74	9.71
7	4.72	4.84	4.47	4.57	4.71	4.68

Filter	0.00	0.00	0.00	0.00	0.00	0.00
Delivered dose (µg)	96.92	98.04	94.70	95.60	96.87	96.56
Amount in spacer (µg)	9.69	9.80	9.47	9.56	9.69	9.66
TED (µg)	106.62	107.85	104.17	105.16	106.56	106.21
FPD (%nominal dose)	64.06	64.68	62.83	63.33	64.03	63.86
MMAD (µm)	2.87	2.81	2.79	2.79	2.78	2.81
GSD	2.01	2.09	2.10	2.07	2.10	2.07

Table 6.9: Aerodynamic particle profile at flows of 50 L/min with an Aerochamber® plus spacer.

Amount deposited on each stage (µg)						
Stage	**Run 1**	**Run 2**	**Run 3**	**Run 4**	**Run 5**	**Mean (µg)**
Induction port	9.55	9.72	9.79	9.68	9.62	9.69
-1	8.72	8.90	8.97	8.86	8.80	8.86
0	7.71	7.89	7.96	7.85	7.79	7.85
1	8.69	8.86	8.93	8.82	8.76	8.83
2	16.59	16.76	16.83	16.72	16.66	16.73

3	19.40	19.58	19.65	19.54	19.48	19.54
4	20.17	20.34	20.41	20.30	20.24	20.31
5	9.57	9.75	9.82	9.71	9.65	9.71
6	2.40	2.58	2.65	2.54	2.48	2.54
Filter	0.00	0.00	0.00	0.02	0.04	0.00
Delivered dose (µg)	102.79	104.39	105.01	104.03	103.50	104.06
Amount in spacer (µg)	10.28	10.44	10.50	10.40	10.35	10.41
TED (µg)	113.07	114.82	115.51	114.43	113.85	114.47
FPD (%nominal dose)	64.01	64.90	65.24	64.70	64.42	64.72
MMAD (µm)	3.07	3.09	3.06	3.04	3.02	3.07
GSD	2.18	2.14	2.16	2.14	2.17	2.16

Table 6.10: Aerodynamic particle profile at flows of 60 L/min with an Aerochamber® plus spacer.

Amount deposited on each stage (µg)						
Stage	**Run 1**	**Run 2**	**Run 3**	**Run 4**	**Run 5**	**Mean (µg)**
Induction port	9.86	10.09	10.24	10.23	10.00	10.07
-1	9.92	5.08	5.15	5.14	5.03	6.72
0	7.02	7.13	7.21	7.20	7.08	7.12
1	10.34	10.46	10.53	10.52	10.41	10.44
2	16.09	16.21	16.28	16.27	16.16	16.19
3	20.06	20.17	20.25	20.24	20.13	20.16
4	16.67	16.79	16.86	16.85	16.74	16.77
5	4.75	4.80	4.84	4.84	4.78	4.80
6	2.61	2.67	2.71	2.70	2.64	2.66
Filter	1.24	1.27	1.29	1.29	1.26	1.27
Delivered dose (µg)	88.21	94.16	93.27	93.84	93.91	91.88
Amount in spacer (µg)	8.82	9.42	9.33	9.38	9.39	9.19
TED (µg)	97.03	103.58	102.60	103.22	103.30	101.07

FPD (%nominal dose)	57.06	61.63	61.19	61.47	61.51	59.96
MMAD (µm)	3.56	3.59	3.53	3.51	3.49	3.56
GSD	2.27	2.21	2.26	2.21	2.24	2.25

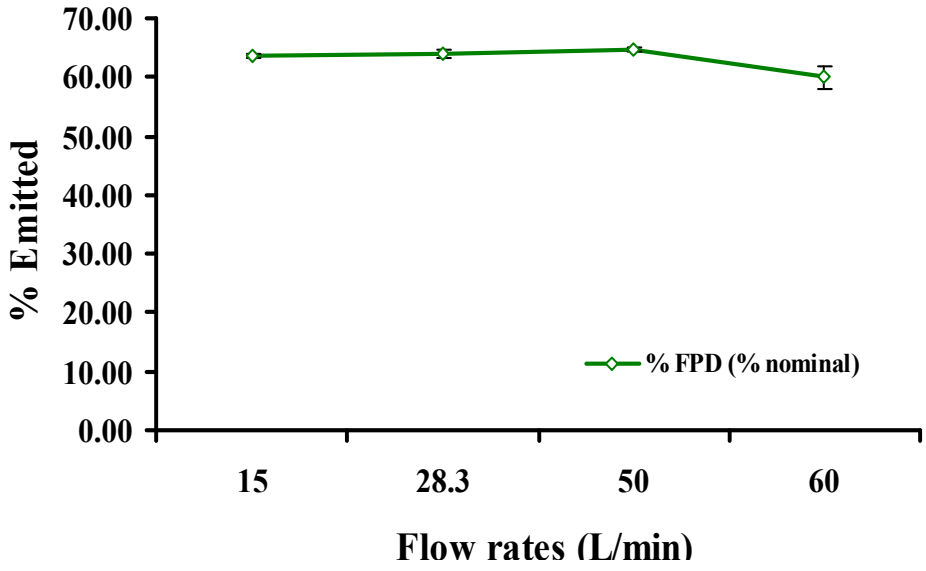

Figure 6.8: Comparison of %FPD (%nominal) at flow rate of 15, 28.3, 50, and 60 L/min with Aerochamber® Plus.

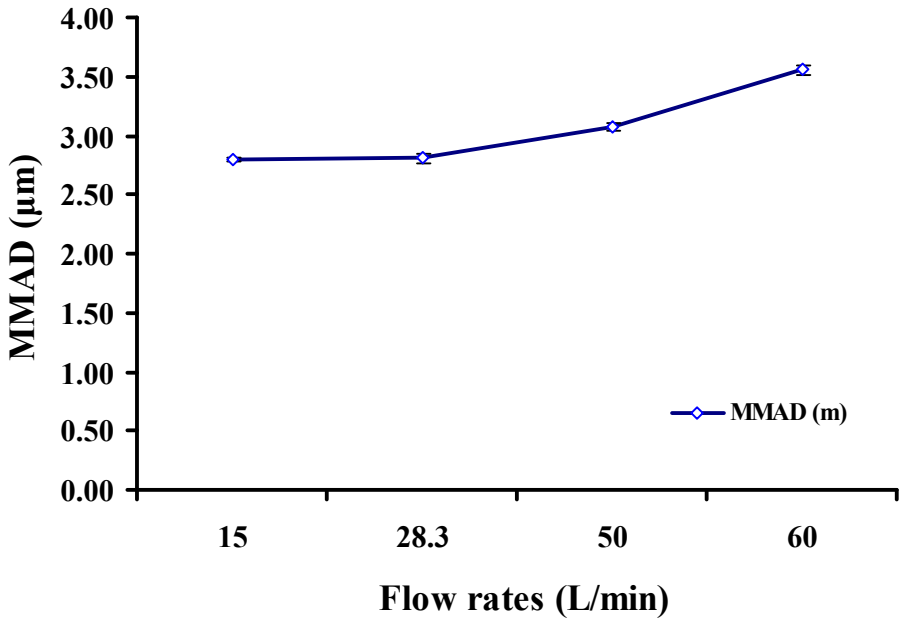

Figure 6.9: Comparison of MMAD (µm) at flow rate of 15, 28.3, 50, and 60 L/min with Aerochamber® Plus.

Table 6.11: Aerodynamic particle profile at flows of 15 L/min with a Volumatic® spacer.

Amount deposited on each stage (µg)						
Stage	**Run 1**	**Run 2**	**Run 3**	**Run 4**	**Run 5**	**Mean (µg)**
Induction port	1.83	1.68	1.79	0.00	0.00	1.77
0	5.61	5.46	5.57	5.49	5.57	5.55
1	4.76	4.61	4.72	4.64	4.72	4.70

2	9.77	9.62	9.73	9.65	9.73	9.71
3	13.70	13.55	13.66	13.58	13.66	13.64
4	19.88	19.73	19.84	19.76	19.84	19.82
5	17.64	17.49	17.60	17.52	17.60	17.58
6	9.64	9.49	9.60	9.52	9.60	9.57
7	4.63	4.48	4.59	4.51	4.59	4.56
Filter	0.00	0.00	0.00	0.00	0.00	0.00
Delivered dose (µg)	87.47	86.13	87.09	84.66	85.31	86.89
Amount in spacer (µg)	8.75	8.61	8.71	8.47	8.53	8.69
TED (µg)	96.22	94.74	95.80	93.12	93.84	95.58
FPD (%nominal dose)	62.72	61.97	62.51	62.11	62.51	62.40
MMAD (µm)	2.71	2.72	2.73	2.70	2.73	2.72
GSD	2.13	2.19	2.18	2.14	2.13	2.15

Table 6.12: Aerodynamic particle profile at flows of 28.3 L/min with a Volumatic® spacer.

Amount deposited on each stage (µg)						
Stage	**Run 1**	**Run 2**	**Run 3**	**Run 4**	**Run 5**	**Mean µg**
Induction port	2.24	2.39	2.13	5.12	5.27	2.25
0	5.92	6.07	5.81	5.82	5.97	5.93
1	5.27	5.42	5.17	5.17	5.32	5.28
2	10.19	10.34	10.08	10.10	10.25	10.20
3	13.86	14.01	13.76	13.79	13.94	13.88
4	20.46	20.61	20.36	20.40	20.55	20.47
5	17.72	17.86	17.61	17.65	17.80	17.73
6	9.25	9.40	9.15	9.17	9.31	9.27
7	4.52	4.67	4.42	4.43	4.57	4.54
Filter	0.00	0.00	0.00	0.00	0.00	0.00
Delivered dose (µg)	89.42	90.77	88.49	91.66	92.97	89.56
Amount in spacer (µg)	8.94	9.08	8.85	9.17	9.30	8.96
TED (µg)	98.36	99.84	97.34	100.83	102.27	98.52
FPD (%nominal	63.33	64.08	62.82	62.95	63.68	63.41

dose)						
MMAD (µm)	2.90	2.90	2.93	2.91	2.92	2.91
GSD	2.18	2.19	2.11	2.13	2.15	2.16

Table 6.13: Aerodynamic particle profile at flows of 50 L/min with a Volumatic® spacer.

Amount deposited on each stage (µg)						
Stage	**Run 1**	**Run 2**	**Run 3**	**Run 4**	**Run 5**	**Mean µg**
Induction port	2.04	2.14	2.29	5.78	5.60	2.16
-1	9.34	9.44	9.59	9.64	9.45	9.46
0	5.73	5.83	5.98	6.02	5.83	5.84
1	8.84	8.95	9.09	9.14	8.96	8.96
2	18.20	18.31	18.45	18.52	18.34	18.32
3	22.60	22.70	22.85	22.93	22.75	22.72
4	17.15	17.25	17.40	17.47	17.28	17.26
5	7.65	7.76	7.90	7.95	7.76	7.77
6	2.50	2.61	2.75	2.79	2.60	2.62
Filter	0.00	0.00	0.00	0.00	0.00	0.00
Delivered dose (µg)	94.05	95.00	96.29	100.24	98.58	95.11

Amount in spacer (µg)	9.40	9.50	9.63	10.02	9.86	9.51
TED (µg)	103.45	104.50	105.92	110.26	108.43	104.62
FPD (%nominal dose)	64.12	64.65	65.37	65.67	64.74	64.71
MMAD (µm)	3.27	3.24	3.29	3.21	3.20	3.27
GSD	2.14	2.17	2.12	2.08	2.11	2.14

Table 6.14: Aerodynamic particle profile at flows of 60 L/min with a Volumatic® spacer.

Amount deposited on each stage (µg)						
Stage	**Run 1**	**Run 2**	**Run 3**	**Run 4**	**Run 5**	**Mean µg**
Induction port	5.04	5.18	5.07	5.98	5.79	5.10
-1	10.31	10.45	10.34	8.53	9.67	10.37
0	9.75	9.89	9.78	9.01	9.95	9.81
1	9.13	9.27	9.54	9.54	9.64	9.31
2	21.08	21.22	21.11	20.72	20.63	21.14
3	25.90	25.73	25.09	25.80	25.01	25.57
4	10.03	10.17	10.06	10.18	10.09	10.08

5	2.04	2.17	2.06	2.18	2.08	2.09
6	2.21	2.34	2.23	2.16	1.17	2.26
Filter	0.00	0.00	0.00	0.01	0.02	0.00
Delivered dose (µg)	95.50	96.41	95.28	94.10	94.05	95.73
Amount in spacer (µg)	9.55	9.64	9.53	9.41	9.41	9.57
TED (µg)	105.05	106.06	104.81	103.51	103.46	105.30
FPD (%nominal dose)	58.66	59.08	58.41	58.82	57.20	58.43
MMAD (µm)	3.56	3.51	3.59	3.47	3.51	3.55
GSD	2.27	2.21	2.26	2.34	2.31	2.25

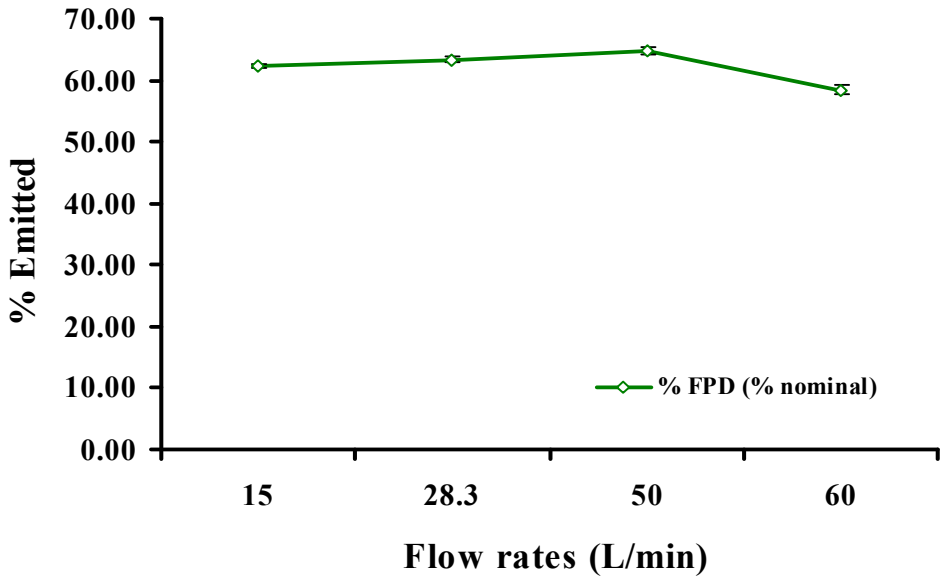

Figure 6.10: Comparison of %FPD and %FPF (%nominal) at flow rate of 15, 28.3, 50, and 60 L/min with Volumatic® Plus.

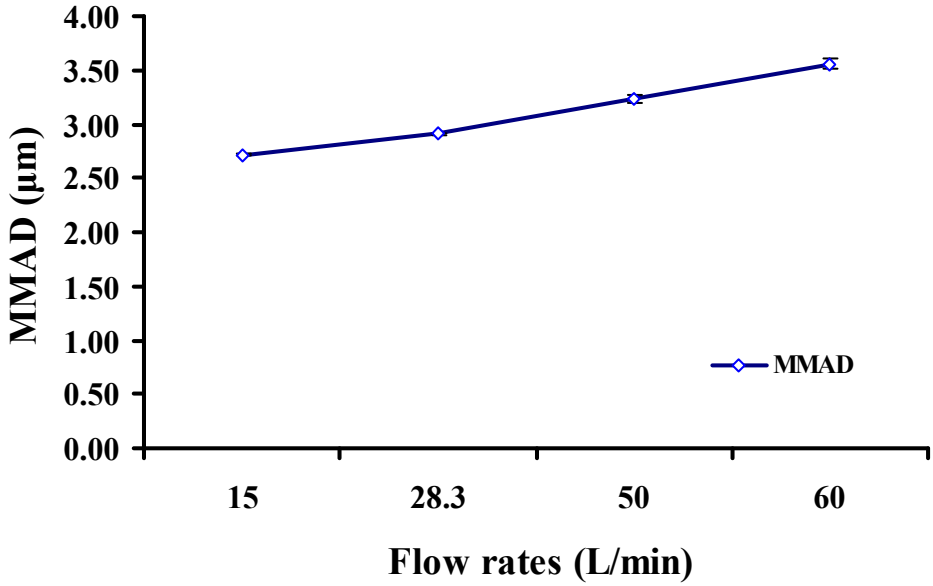

Figure 6.11: Comparison of MMAD (µg) at flow rate of 15, 28.3, 50, and 60 L/min with Volumatic® Plus.

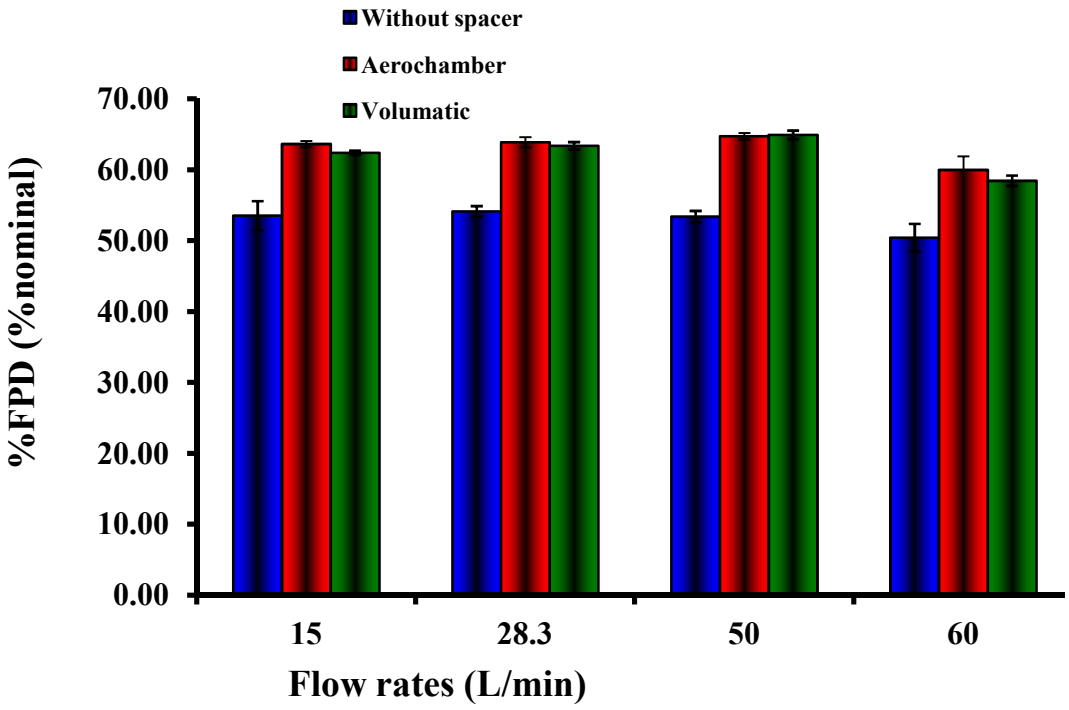

Figure 6.12: Comparison of FPD with and without use of spacers.

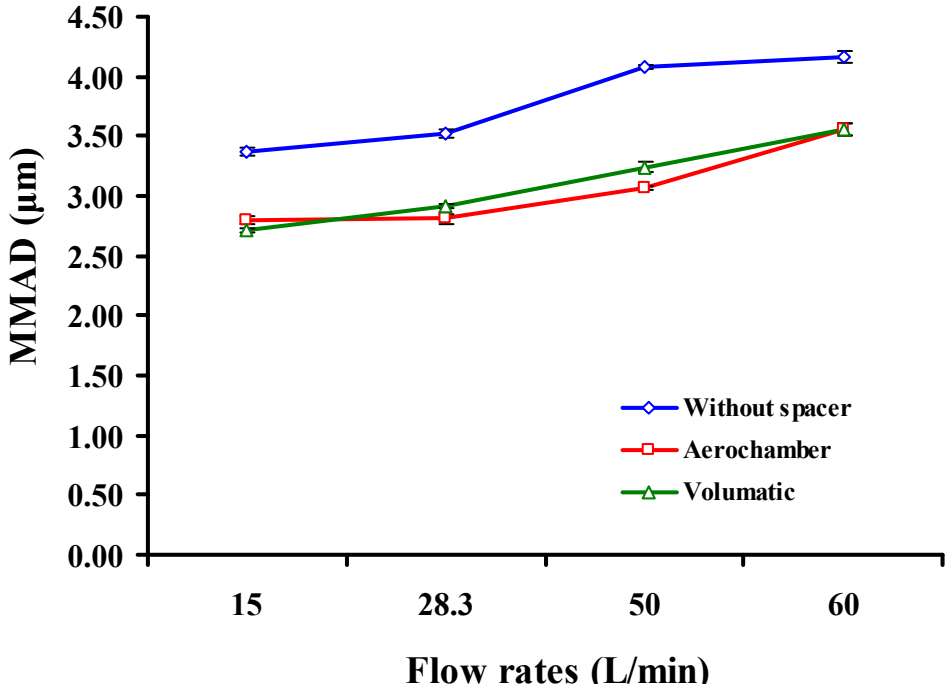

Figure 6.13: Comparison of MMAD (µm) with and without the use of spacers.

Table 6.15: Statistical comparison of the MMAD values with and without the use of spacer. *p<0.05, **p<0.01, ***p<0.001 taken as a significant differences.

	MMAD (μM) 15 L/min	MMAD (μM) 28.3 L/min	MMAD (μM) 50 L/min	MMAD (μM) 60 L/min
Aerochamber vs No spacer	***p<0.001	**p<0.01	***p<0.001	***p<0.001
Volumatic vs No spacer	**p<0.01	**p<0.01	***p<0.001	***p<0.001
Aerochamber vs Volumatic	p=0.640	p=0.072	p=0.514	p=0.779

Table 6.16: Statistical comparison of the effect of varying inhalation flows on %FPD (%nominal) without using spacer and with Aerochamber Plus® and Volumatic® spacers. p<0.05, p<0.01, p<0.001 taken as a significant differences.

Inhalation flow rate (L/min)	**Without spacer** %FPD (% nominal) p-values (95% confidence interval)	**Aerochamber Plus®** %FPD (% nominal) p-values (95% confidence interval)	**Volumatic®** %FPD (% nominal) p-values (95% confidence interval)

15 vs 28.3	p=0.125	p=0.345	p=0.345
15 vs 50	p=0.0504	p=0.903	p=0.218
15vs 60	p=0.618	p=0.357	p=0.125
28.3 vs 50	p=0.953	p=0.367	p=0.758
28.3 vs 60	p=0.111	p=0.09	p=0.525
50 vs 60	p=0.125	p=0.106	p=0.740

6.4 Discussion

In vitro measurements of total emitted dose, the fine particle dose and the aerodynamic particle size distribution are quality control measures to assess inhaled products and demonstrate the accuracy and consistency of output from the inhalation device. In addition to quality assurance, these measurements may also be predictive of the in-vivo performance of inhaled products. In line with this, studies have shown that there is a correlation between the emitted dose, especially fine particle dose, lung deposition, clinical effect, and inhalation rate used (Tarsin et al, 2002, Borgstorm et al, 1994).

Targeted delivery of inhalation products into the lungs is achieved by means of three types of inhalation devices, the pressurised metered-dose inhalers (MDI) and dry powder-inhalers (DPI), and nebulisers. For environmental reasons, the

chlorofluorocarbon (CFC) propellants used in MDI are now been replaced by ozone friendly hydrofluoroalkanes (HFA). These new generation of MDI have been developed to provide effective lung deposition of the drug particles and have a favourable safety and tolerability profile. However, HFA-based formulation for MDI presents particular technical difficulties especially in terms of ensuring dose content uniformity (Acerbi et al, 2007).

In line with this Modullite® technology was introduced to address some of HFA-based formulation drawbacks. Modulite® technology allows the development of HFA solution formulations that can mimic the established CFC-based drug formulations and provide formulations with novel particle size distributions by manipulation of the aerosol clouds and particle size. In this context, Modulite® technology has achieved a successful transition from CFC to HFA solution MDI for drugs such as budesonide (Vastagh et al, 2003) and formoterol fumarate (Houghton et al, 2004).

In the present study, the dose emission of formoterol fumarate from an Atimos® Modullite® was investigated at inhalation rates of 10, 28.3, 60, and 90 L/min whilst using different inhalation volumes of 2L and 4L. Results from this study demonstrated that the emitted dose of formoterol fumarate from an Atimos® Modullite® was consistent across a range of inhalation flow rates 10-90 L/min. The study also produced comparable results using inhalation volumes of 2L and 4L. This is in accordance with studies reported by Malton et al (1996) that have shown no flow dependency properties of MDI-containing terbuline.

Similarly, studies assessing the effect of inhalation flow rate on the dosing characteristics of DPI and MDI products demonstrated that in all cases, independent of drug or device used, the MDI products had a more reproducible respirable dose than DPI products tested (Ross and Schultz, 1996).

Previous 2-D scintigraphic studies to assess the lung deposition of 99m-technetium (Tc)-radiolabeled formoterol fumarate after inhalation in healthy volunteers, asthmatic and COPD patients showed that formoterol from Atimos® and Forair® Modulite provides high and homogenous drug distribution in the lung (Acerbi et al, 2005).

MDI devices have been reported to demonstrate more reproducible dosing characteristics (Ross and Schultz, 1996). This dose consistency is a function of proper shaking before actuation, but the dosing may become erratic, especially towards the end of the life of the canister. Moreover, Modulite® technology ensures stability and consistency of the formulation throughout the life of the MDI canister. For instance, when drug delivery performance of a Modulite® formulation of formoterol was investigated, formoterol delivery remained uniform and was within European Pharmacopeia specifications (European Directorate for the Quality of Medices, 2002). The data provided a picture of inter and intra inhaler dosing variability within defined inhalation flows. Moreover, although the present data showed that the doses emitted by the MDI Atimos® Modulite® at different inhalation flows were not significantly different, there was a tendency for lower flow rates to result in similar dosing which may provide higher deposition of the drug with Atimos Modulite®. Previous studies by Newman (1981) investigating lung deposition of budesonide and terbutaline in relation to the inhalation flow achieved by patients when inhaling through a MDI device. The results showed that the optimum inhalation flow for the use of MDI was less than 28.3 L/min.

Both the dose delivered from the device and the particle sizes of the medication are important parameters for inhalation products because they influence the amount of drug that is delivered to the patient's lung. The key parameters affecting efficacy of MDI is

the aerodynamic particle size distribution of the drug particles delivered to the patient each time the dose is actuated, including patient's breathing pattern, the inspiratory flow rate, and the hand-mouth coordination (Hoe et al, 2009).

In order to study the effects of inhalation flow rates on the particle size distribution of formoterol fumarate from an Atimos Modullite®, a multistage cascade impactor was used.

The present study demonstrated that although the fine particle dose decreased with increasing inhalation flow rate from 15 to 60 L/min, this was not statistically significant. Results also showed that the MMAD values increased with increase in the inhalation flow rate. This data is in agreement with studies carried out by Karen et al (1998) where no differences were observed in dose emission characteristics of MDI when inhalation flow rates increased from 30 to 55 and 80 L/min.

However, some reported studies also emphasise on the need for slow and deep inhalation flow rates whilst inhaling through a MDI. Studies by Newman et al (1991) have shown that low inhalation flow rates of 30 L/min were more beneficial to patients. In separate studies by Bennett et al (1987) and Dolovich et al (1981) it was demonstrated that increasing inhalation flow rate resulted in a decrease in the lung deposition and penetration into peripheral airways of the lung. Faster inhalation of 60L/min resulted in reduced peripheral deposition because aerosols were more readily deposited in the oropharyngeal region. The decrease of the fine particle dose with increase in flow rate may be due to higher impact of the drug in the induction port and spacer at higher flow rates.

Another crucial obstacle in the delivery efficiency of an MDI depends on hand-mouth coordination. Crompton et al (1982) found that 51% of patients experienced problems coordinating actuation of the device with inhalation, 24% of patients halted inspiration

upon actuation of the aerosol into the mouth, and 12% inspired through the nose instead of the mouth when the aerosol was actuated into the mouth. As a result a number of different spacer tubes, valved holding chambers, and mouth extensions have been developed to eliminate coordination requirements. These extensions reduce the below-freezing spray temperature effect that causes some patients to stop inhaling. Additionally, they reduce the particle size distribution as the aerosol velocity is reduced hence reducing the amount of drug deposited in the oropharynx.
In line with this, the performance of the AeroChamber® Plus and Volumatic® spacers in delivering formoterol fumarate from an Atimos® Modulite MDI was compared with results obtained from using the MDI alone. Both devices were washed in detergent, rinsed with cold water and allowed to air dry according to the manufacturer's instructions. However, Pierart et al (1999) showed in their comparison of various pre-treatment techniques that rinsing after washing in detergent is not as effective as washing and drip-drying without rinsing. Present results demonstrated that the fine particle dose emission of formoterol fumarate from the MDI device with either a Volumatic® or AeroChamber® Plus spacer was significantly ($P<0.01$) higher than that of MDI alone. This is due to the fact that spacer device reduces the primary droplet size by providing extra time for complete evaporation of propellant and reduces the velocity of the aerosol particles passing through the device (Moren, 1978, Vidgren et al, 1987). Consequently, large particles will impact on the holding chamber walls significantly reducing the drug aerosol available for inhalation from plastic spacers hence reducing deposition of these large particles in the oropharyngeal region. The ability of holding chambers to efficiently deliver drug particles can sometimes be compromised, particularly by the presence of electrostatic charge on the inner walls of the chambers. This has been reported recently in studies by Perart et al (2003) and Glover and Chan (2004) who demonstrated that the electrostatic charge is more prevalent with some

propellants such as the new hydrofluoroalkane (HFA) intended as a replacement for chorofluorecarbon (CFC) propellants.

The fine particle dose (%FPD) at different flow rates using the Aerochamber® Plus and Volumatic® spacers were also compared. At very low inhalation flow rate of 15 L/min the %FPD using Aerocamber Plus® and Volumatic® spacers were 63.62% (0.44) and 62.40% (0.31) respectively as compared to higher flow rate of 60 L/min where values were 59.96% (1.97) and 58.43% (0.73), respectively. Although the difference in fine particle dose between the two flow rates was not significant, these results indicate that at very low flow rates more percentage of fine particle (<5 μm) gets deposited in the lungs thereby may provide an improved clinical response in patients with lung disorders.

Moreover, low flow rates of 15 L/min have produced the highest fine particle dose and the lowest MMAD. This indicates that this flow rate would produce the better lung deposition compared to high flow rate when using Aerochamber® Plus or Volumatic® spacers and without spacers. Furthermore, a decrease in MMAD values suggests that such holding chambers produce finer aerosol particles, which are more uniformly distributed and therefore increase the delivery of the drug to the peripheral airways of the lungs.

Results also demonstrate no significant differences in MMAD between the AeroChamber® Plus and Volumatic® Plus spacers. These results are in agreement with those reported by Coppolo et al (2006) as they also found no significant differences in the performance of the Aerochamber® Plus and Volumatic® spacers in delivering HFA-propelled salbutamol.

Several studies have investigated the clinical efficacy of spacers compared with that of MDI alone (Konig, 1985, Newman and Newhouse, 1996). In terms of broncholidation,

some studies suggest that the use of spacers do not confer any additional benefit when MDI alone is correctly used. In contrast, other studies show that compared with MDI alone, spacers may actually enhance bronchodilation (Dolovich et al, 1983, Lee and Evans, 1987, Tobin et al, 1982, Pedersen et al, 1983). In the present study, the use of spacer has generally improved the amount of fine particle dose emitted through MDI.

The main reasons that seem to account for this discrepancy are the inclusion of patients with poor inhalation techniques in the studies, dissimilarities in the types of inhalation devices, and the characteristics of the patients studied. Recently, the effects of a β2-adrenergic bronchodilator through a MDI alone and two different spacers (Volumatic and Jet Smallvolume spacer) on the magnitude of and the velocity of large and small airways bronchodilator response were compared in asthmatic patients who correctly operate a MDI. It was observed even in patients with good inhalation techniques, both spacers enhanced bronchodilation compared with MDI alone (Fontana et al, 1999). This comparative study was aimed at ascertaining whether differences exist in the clinical response to administration of inhaled medication via MDI plus spacer or MDI alone. In the present study a similar approach was adopted and results recommend that patients having problems using the MDI should be prescribed with spacer such as Aerochamber® Plus and Volumatic® in order to improve lung deposition of the medication.

6.5 Conclusion

Dose content uniformity of MDI products was evaluated and it was found that consistent doses are obtained through the life of the MDI inhaler devices. Furthermore, the effect of flow rates was assessed and total emitted dose was consistent across the different flow rates. Additionally, there was no difference between 2 L and 4 L inhalation volume.

Overall the mean doses for all flow rates were within the acceptable range of pharmacopeia.

On comparing the results for the influence of inspiratory flow rate on the aerosol particle size distribution from MDI inhalers, it was concluded that at very low flow rates Atimos® Modulite shows higher fine particle dose which indicates that a patient is inhaling at very low flow rate may achieve a good lung deposition. This study also shows that the use of spacer devices will enable the patients to have good hand to lung coordination thereby providing better lung deposition.

CHAPTER SEVEN

GENERAL DISCUTION AND CONCLUSION

7. General Discussion and Conclusion

Formoterol fumarate is a selective and potent long acting beta2 adrenoreceptor agonist which is recommended for the management of asthma, the prevention of exercise-induced bronchospasm, and COPD. Formoterol is available in MDI, marketed as Atimos Modulite® (Trinity Chiesi) and DPI, marketed as Foradil Aeroliser® (Norvatis), Oxis Tubuhaler® (AstraZeneca) and Easyhaler® (Orion Pharma).

Important parameters for inhalation product performance are the amount of medication delivered to the patient and the effective aerodynamic particle size of the medication. DPI and MDI are two major inhalation systems. To estimate the amount of drug delivered to the lungs a combination of the total emitted dose and particle size are essential. Furthermore, because inspiratory flow rate may vary from dose to dose in a given patient and between patients its effect on inhalation device performance and hence the potential to influence the clinical results must also be evaluated. Additionally, lung disease may also change the deposition characteristics of drug particles in the lungs.

Due to the variety of parameters determining drug deposition into the lungs, the efficacies of such devices were determined in-vitro. Firstly, the present study was designed to determine the formoterol fumarate dose emitted from an Atimos® Modulite® using peak inhalation rates of 10, 28.3, 60, and 90 L/min that correspond to normal patient use and with inhalation volumes of 4L and 2L. The study also included the evaluation of the uniformity of dose emitted from the Atimos® Modulite® and to identify inter and intra inhaler dose emission variability from different batches of Atimos® Modulite® devices.

Results revealed that the emitted dose of formoterol fumarate from an Atimos® Modullite® was consistent across a range of inhalation flow rates 10-90 L/min. In

addition, the study also produced comparable results using inhalation volumes of 2L and 4L.

Moreover, the aerodynamic properties of dose emitted from an Atimos Modulite® with and without spacer devices at flows of 15, 28.3, 50, 60 L/min using mixing inlet at inhalation volume of 4L were also determined.

Overall the mean nominal doses for all flow rates were within the acceptable range of pharmacopeia. On comparing the results for the influence of inspiratory flow rate on the aerosol particle size distribution from MDI inhalers, it was concluded that inspiratory flow rate does not influence the emitted fine particle dose through Atimos Modullite®. Nonetheless, low flow rates Atimos® Modulite shows higher fine particle dose and a significant ($p<0.001$) increase in MMAD values as inhalation flow rates increased, which indicates that a patient inhaling at low flow rate will achieve a good lung deposition.

The use of spacer devices demonstrated to improve the amount of fine particle dose being generated through Atimos Modulite®. Such results indicate that patients with poor inhalation technique will mostly benefit from the use of a spacer device compared to patients that show good hand to lung coordination. It is thought that the high velocity of aerosol spray whilst inhaling through Atimos Modulite® with a spacer device will be reduced hence reducing the amount of drug deposited in the oropharynx thereby providing better lung deposition.

Secondly, the present study was designed to identify and compare the dose emission of formoterol from three DPI devices (Foradil Aeroliser®, Easyhaler®, Oxis Turbuhaler®)

at varying inhalation flow rates using inhalation volumes of 4 L and 2 L and one and two inhalations per single dose.

The (SD) mean emitted dose through Foradil Aeroliser® at low inhalation flow rate of 20 and 28.3 L/min (4 L) was 59.42 (0.53) % and 73.13 (0.81) %, and at high inhalation flow rates of 60 and 90L/min (4 L) was 80.97 (0.42) % and 88.09 (0.57) %, respectively.

The (SD) mean emitted dose through Easyhaler® at low inhalation flow rate of 20 and 28.3 L/min (4 L) was 71.63 (1.84) % and 85.38 (3.91) % and at high inhalation flow rates of 40 and 60 L/min (4 L) was 87.89 (1.11) % and 90.15 (3.49) %, respectively.

The (SD) mean emitted dose through Oxis Turbuhaler® at low inhalation flow rate of 20 and 28.3 L/min (4 L) was 57.44 (0.88) % and 63.65 (1.57) % and at high inhalation flow rates of 40 and 60 L/min (4 L) was 67.07 (1.34) and 73.16 (2.62) %, respectively.

These results demonstrate that a greater formoterol dose emission is achieved through both Easyhaler® and Foradil Aeroliser® devices compared to Turbuhaler. In addition, a more effective dose emission was achieved at inhalation volumes of 4 L compared with inhalation volume of 2 L in all three devices.

Moreover, the different inhalation flow rates have also been shown to have a significant effect on dose delivery. The data on all three DPI devices (Foradil Aeroliser®, Turbuhaler®, and Easyhaler®) inhalation performance show a tendency for lower flows to produce reduced dosing and lower deposition of drug. Subsequently, as the flow rate increases higher dose emission was obtained.

This is indeed a common pattern when inhaling through DPI devices. Since the energy created inside a DPI to generate the respirable dose is a product of inhalation flow and the inhaler's internal resistance, hence the faster the inhalation flow rate the higher will

be the energy. Although patients are instructed to inhale as fast as they can and for as long as they can, for some patients inhaling too fast may result in high oropharyngeal impaction and low lung deposition. This is particularly an issue when inhaling from low resistance device such as Foradil Aeroliser®, where the inspiratory effort required to generate high flows are relatively lower compared to that of a high resistance device. Nonetheless, the particle loss in airways by impaction at higher air flows is expected to be less for the better dispersed drug particles.
On the other hand, some patients suffering from pulmonary disease may not be able to inhale fast through the device. This is particularly problematic when inhaling through high resistance devices such as Oxis Turbuhaler®.

Interestingly, Easyhaler® although a high resistance device, its design was focused on minimising the flow-dependent dose emission that occurs with the Turbuhaler® hence allowing improved dose emission at lower inhalation flow rates.

In line with this, the present data demonstrated that by inhaling twice from each single dose through Foradil Aeroliser® miximises the dose emission and ensures effective emptying of the capsule content. Turbuhaler® performance showed the need to inhale twice only when the inhalation volumes are critical (2 L). Easyhaler® performance showed the need to inhale twice only at higher flow inhalation rates of 40 and 60 L/min.

Thirdly, the present study was designed to identify and compare lung deposition and particle size distribution of formoterol from three DPI devices (Oxis Turbuhaler®, Foradil Aeroliser®, Easyhaler®) at varying inhalation flow rates using inhalation volumes of 4 L and 2 L. This was performed using Anderson Cascade Impactor with mixing inlet valve. The study also focused on determining the total drug delivery after one and two inhalations from each dose for all three DPI devices.

The present data demonstrated that at a relatively low flow rate of 28.3 L/min the mean total emitted dose (SD) at one inhalation and using inhalation volume of 4L from Turbuhaler®, Aeroliser®, and Easyhaler® were 63.66% (1.57), 68.18% (0.76) and 85.38% (3.91), respectively. Furthermore, at higher flow rates of 60 L/min the mean total emitted dose (SD) from Turbuhaler, Aeroliser, and Easyhaler were 73.16% (2.62), 82.33% (0.60), and 90.15% (3.39), respectively.

The results of MMAD showed a significant ($p<0.01$-$p<0.001$) decrease in particle size for all three DPI devices as inhalation flow rates increased.

Again it is observed that due to the differences associated with each DPI devices, especially the different intrinsic resistance, Turbuhaler ® delivered less formoterol aerosol than Aeroliser® and Easyhaler®.

The present data revealed that there were no significant differences in both nominal dose and fine particle dose of formoterol from Turbuhaler following one and two inhalations at flow rates of 10-60 L/min using 4 L inhalation volume. At inhalation volume of 2 L the nominal dose and fine particle dose from two inhalations were significantly ($p<0.05$) higher at inhalation flow rates of 10-60 L/min compared to one inhalation.

The nominal dose and fine particle dose of formoterol from Aeroliser following two inhalations increased significantly ($p<0.01$-$p<0.001$) with increase in inspiratory flow rate at both 4 L and 2 L inhalation volumes. Whereas the nominal dose and fine particle dose of formoterol from Easyhaler® following two inhalations was significantly higher ($p<0.05$) at higher flow rates of 40 and 60 L/min compared to one inhalation, but there was no significant difference at lower flow rates of 10, 20, and 28.3 L/min.

These finding correlated with data reported on formoterol dose emission achieved through Easyhaler ®, Foradil Aeroliser® and Turbuhaler®.

Therefore, due to design differences in DPI devices, fine particle dose delivered from DPI devices was shown to be dependent not only on inhalation flow rates following a specific inhalation effort but the specific intrinsic resistance of each DPI device. As a result low resistance DPI devices such as Aeroliser® delivered higher fine particle dose compared to high resistance Turbuhaler® with the same inhalation effort. Interestingly, Easyhaler®, a relatively high intrinsic resistance DPI device delivered the highest fine particle dose of formoterol with the same inhalation effort.

The present data suggest that although fast inspiratory flow is required in order to produce high fine particle dose, the use suggests that young children and those with severe obstructive disease are more likely to have problems using a fast inhalation flow. However, low resistance devices such as Aeroliser® seems to be highly favourable as they deliver higher fine particle dose at lower flow rates. In addition, the use of two inhalations per single dose through Foradil Aeroliser® seems equally favourable.

Conversely, Turbuhaler is less favourable for those with severe obstructive disease as it requires fast inhalation flow in order to deliver optimal fine particle dose. However, for those with critical inhalation volume (2 L) inhaling twice through Oxis Turbuhaler® may prove beneficial.

Interestingly, Easyhaler® also seems to be highly favourable as it delivers high fine particle dose at relatively low inhalation flow rates. Additionally, at higher flow rates the use of two inhalations significantly increases fine particle dose delivery. This is indeed desirable, especially for young children, COPD and asthma sufferers, elderly, and those who have poor lung function who may not be able to generate fast inhalation flows.

Future Work

The data generated from this thesis have shown that total dose emission from an AtimosModulitte® is less affected by inhalation flow rate and inhalation volume compared to DPI devices including Turbuhaler®, Easyhaler® and Aeroliser®.

Data from Turbuhaler® in particular revealed that patients with low inspiratory capacity may receive a low dose which in turn might not provide the required therapeutic target.

Therefore, it would be useful to extend these in vitro findings to clinical studies using patients with low inspiratory capacity. It would be interesting to carry out in vivo relative lung bioavailability and lung deposition of formoterol to show whether oropharyngeal deposition from the different DPI devices (Turbuhaler®, Easyhale® r, and Aeroliser®) may be influenced by inhalation flow rate as shown in-vitro.

Also, present results have shown that patients need to adapt correct inhalation techniques to generate an emitted dose with the appropriate fine particle dose for lung deposition. Another important factor is the inhalation formulation. Micronization is generally used for the preparation of the DPIs formulation. However, this process relies on particle-particle collision to reduce the particle size and hence particles may carry high charge which results in lower lung deposition. There are other engineering methods such as Supercritical Fluids (SEDS), Spray Drying which could be used to produce particles with less surface energy and more controlled particle size distribution so it is suggested to extend this further to investigate the potential of these particle engineering methods for the formulation of formoterol in DPIs and to compare the in-vitro characteristics of the different formulations, to each other and to a micronized DPI formulation and then to identify whether it would affect lung deposition.

CHAPTER EIGHT

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